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GLUTATHIONE VALUES OF CORNISH LETHAL AND CREEPER EMBRYOS COMPARED WITH NORMAL SIBS

By

P. W. GREGORY, V. S. ASMUNDSON, HAROLD GOSS
University of California, Davis, California
AND
WALTER LANDAUER
Storrs Agricultural Experiment Station, Storrs, Connecticut

(*Received April 16, 1939*)

INTRODUCTION

During the past six years we have presented considerable evidence that within a species the glutathione concentration of body tissues is somehow correlated with the processes of growth and with the final adult body weight of the races in question. This relationship was first demonstrated in newborn rabbits from races differing in adult weight, Gregory and Goss ('33a, '33b). Later comparative studies showed a similar relationship in chicken embryos and early-hatched chickens, Gregory, Goss, and Asmundson ('36, '37). Goss and Gregory ('35a) have also demonstrated in the rabbit that if genetic constitution for adult weight is held constant and if growth is regulated by food intake (nursing), the rabbits receiving food have a much greater concentration of glutathione in their body tissues than their fasting litter-mate controls. Food and fasting likewise influence the glutathione concentration of the body tissues of newly hatched chickens, Goss, Gregory, and Asmundson (unpublished data). With rats it was possible to demonstrate that if growth is induced by means of the hypophyseal growth hormone for several days, the amount of glutathione in the muscle tissue increases significantly along with the stimulation of an increase in body weight, Gregory and Goss ('34). As Goss and Gregory ('35b) found in the rat, the injection of a small amount of potent hypophyseal growth hormone will markedly reduce the glutathione content of the liver and will slightly reduce the glutathione content of muscle at 8, 12, or 24 hours after injection. These results can be interpreted on the basis of the results of Schaffer and Lee ('35), who presented evidence that the hypophyseal growth hormone is a specific stimulant to protein anabolism. Because of this anabolic activity the non-protein nitrogen and the peptide amino nitrogen of the

tissues decrease after injection with hypophyseal growth hormone. According to Schaffer and Lee, the non-protein nitrogen and the peptide amino nitrogen (which includes glutathione) are synthesized into protein under the influence of the hypophyseal growth hormone. If rats are injected with the hypophyseal growth hormone over a period of several days or weeks, the consumption of food is increased, Gregory and Goss ('34), and the intake of food substances necessary for synthesizing glutathione and protein is increased.

These results, together with studies by other investigators, many of which have been cited in our previous publications, clearly indicate that glutathione is definitely correlated in some manner with the processes of growth and with increase in body size. It should be emphasized that the correlation of glutathione in body tissues with increase in body weight holds for the three conditions that we have investigated: (1) differential growth caused by genetic constitution for adult size, (2) conditions under which genetic constitution for adult size is held constant and growth is controlled by food intake (nutrition), and (3) keeping the genetic constitution constant and provoking growth by means of an hypophyseal preparation.

In all our previous comparative glutathione studies of races of rabbits and chickens differing in genetic constitution for adult size, the genes responsible for the respective adult weights of the several races within a species were quantitative in nature. We wished to determine what effect (if any) certain specific, sharply segregating genes, which suppress the growth rate and consequently reduce the final adult weight, had on the concentration of glutathione in the body tissues at definite periods of development. The two genes selected for this study were Cornish lethal and Creeper. Landauer ('35b) reported the origin, mode of inheritance, and general characteristics of the Cornish lethal mutant. It is characterized as deformed, with abnormal, short extremities, a short, broad head, and bulging eyes. Cornish lethal embryos grow more slowly than normals and die about the time of hatching. In the heterozygous state the legs are somewhat reduced in length.

The Creeper mutant was reported by Cutler ('25). Landauer and his associates have reported on the genetics and on the morphological and physiological expressions of the creeper gene during development, Landauer and Dunn ('30), and Landauer ('32, '34, '35a). Creeper is lethal in the homozygous state. There is a general retardation of

development, ending in death at about the end of the third day of incubation, although hybrid vigor or the specific action of other genes may extend the life beyond the typical lethal period at 72 hours. Creeper in the heterozygous state is only slightly subnormal in survival and cannot be differentiated from normal sibs until the seventh day of incubation. Body weights of heterozygous Creeper and normal embryos are about the same throughout the incubation period. After the fourth week of post-hatching life, however, Creeper chicks have a slight but definitely lower body weight. The long bones of heterozygous Creepers are reduced in length.

EXPERIMENTAL RESULTS

The Cornish lethal.

The stock used to provide embryos for this study was supplied by Landauer. Most of the birds were hybrids or segregates from crosses between the original Cornish stock which carried the lethal, and White Leghorns. Birds previously tested and proved to be heterozygous for the Cornish lethal were shipped to California, where they were maintained in the Poultry Plant at Davis throughout the investigation. All the results reported herein were obtained between July, 1937, and October, 1938. All the eggs were weighed when collected and were then accumulated for about two weeks and incubated. Since the parents were heterozygous for Cornish lethal and since segregation was taking place, the eggs could not be paired until the embryos were removed from the eggs. The Cornish lethal embryos were then paired with homozygous normal or heterozygous normal embryos. In pairing the embryos the original egg weight was always taken into account so that the weight of eggs producing an individual pair (one normal and one Cornish lethal embryo) would be approximately the same. Most of the eggs were paired within five-tenths of a gram. The individual eggs

TABLE 1
SUMMARY OF ALL THE DATA OBTAINED FOR CORNISH LETHAL AND NORMAL EMBRYOS

		Normal Means			Cornish Lethal Means		
Days of incubation	Number of pairs	Embryo wt. in grams	GSH mg. %	Ascorbic acid mg. %	Embryo wt. in grams	GSH mg. %	Ascorbic acid mg. %
14	31	10.0	51.6	25.8	9.6	47.9	28.3
16	19	15.1	44.1	16.6	13.7	38.8	14.7
17	29	18.0	36.7	17.0	15.3	32.5	16.4
18	7	19.8	26.3	9.4	14.5	20.1	10.0
19	24	25.3	39.3	8.0	20.8	38.5	8.0

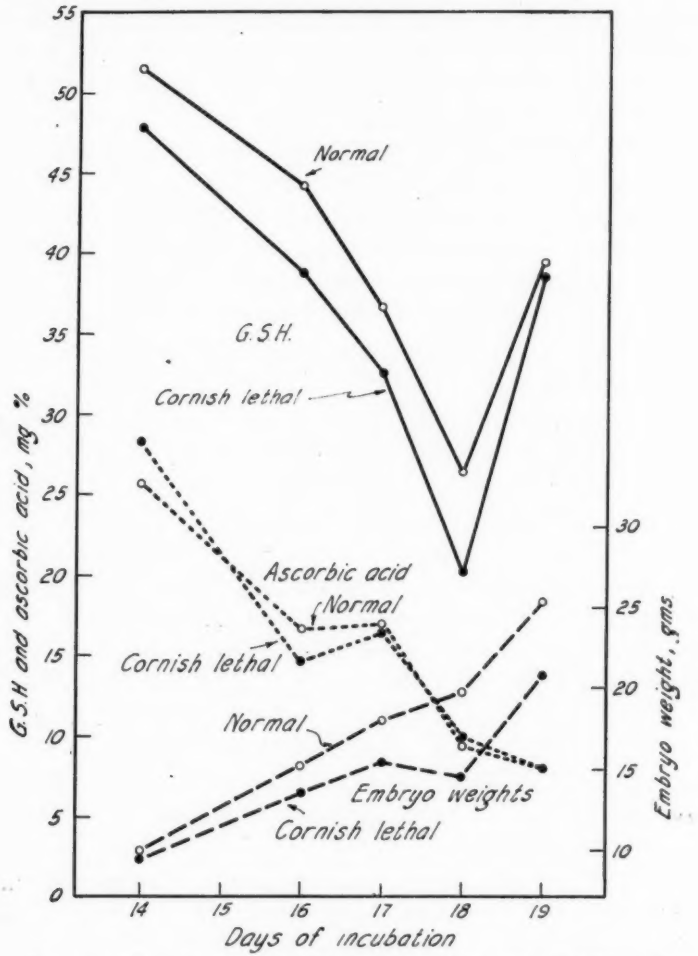

FIG. 1. Curves showing weights, ascorbic acid and glutathione for Cornish normal and Cornish lethal embryos from 14 to 19 days of incubation. Data from table 1.

constituting a pair were placed in the same incubator at the same time, although several different incubations might be necessary to obtain sufficient pairs for a given incubation age.

The mean values, for each incubation age, of all the essential data concerning the Cornish lethal embryos and the normal sibs with which they are paired are summarized in table 1. Figure 1 presents

TABLE 2
SIGNIFICANCE OF MEAN VALUES OF CORNISH LETHAL AND NORMAL EMBRYOS

	t value	t value to be significant	t value to be highly significant
14 days of incubation			
1. GSH, normal vs. Cornish lethal	2.962	—	2.750
2. Weight, normal vs. Cornish lethal	1.674	2.457	—
3. Ascorbic Acid, normal vs. Cornish lethal	1.920	2.457	—
16 days of incubation			
4. GSH, normal vs. Cornish lethal	5.606	—	2.878
5. Weight, normal vs. Cornish lethal	3.130	—	2.878
6. Ascorbic Acid, normal vs. Cornish lethal	1.895	2.552	—
17 days of incubation			
7. GSH, normal vs. Cornish lethal	2.581	2.468	2.763
8. Weight, normal vs. Cornish lethal	4.283	—	2.763
9. Ascorbic Acid, normal vs. Cornish lethal	0.589	2.468	—
18 days of incubation			
10. GSH, normal vs. Cornish lethal	1.633	2.998	—
11. GSH, normal vs. Cornish lethal (one pair omitted — see text)	6.544	—	3.707
12. Weight, normal vs. Cornish lethal	2.887	2.998	—
13. Ascorbic Acid, normal vs. Cornish lethal	0.45	2.998	—
19 days of incubation			
14. GSH, normal vs. Cornish lethal	0.701	2.50	—
15. Weight, normal vs. Cornish lethal	7.254	—	2.807

graphically the same data, taken from table 1. Table 2 shows the significance of all the *t* values for glutathione, weight, and ascorbic acid between Cornish lethal and normal for each incubation age. It should be clearly understood that the data summarized in tables 1 and 2 are taken from the same source and that table 1 gives the number of pairs and the mean values for weight, glutathione, and ascorbic acid for a given period, whereas table 2 gives the *t* values for weight, glutathione, and ascorbic acid for the corresponding period. The weights of Cornish lethal embryos compared with normal sibs show clearly that the Cornish lethal group is retarded during the incubation period (fig. 1, tables 1 and 2). At 14 days the normal embryos are slightly heavier, though the difference is hardly significant. Beyond the 14-day period, differences in weight are definite, becoming more divergent as the age of incubation increases.

The methods used in determining glutathione and ascorbic acid were those described in our last report, Gregory, Goss, and Asmundson ('37). All glutathione values are expressed in milligrams per hundred grams of weight. The glutathione curves for Cornish lethal and for their normal sibs are similar, but the mean values of the Cornish lethal embryos are consistently lower at each period (fig. 1 and tables 1 and

2). These differences are statistically significant for all the incubation periods except the nineteenth day.

Because of limited material only seven pairs of embryos were analyzed at the 18-day period. When these seven pairs are considered, the t value is not significant even though the difference between the means of the groups is appreciable. Among the individual pairs is one in which the glutathione value of the Cornish lethal embryo greatly exceeds that of the normal Cornish embryo with which it is paired. The weight of the normal embryo of the pair is only 13.3 grams, and this weight is discontinuous with weights of the other embryos of the same age for the group. The glutathione value of this exceedingly light embryo is also low and discontinuous with the others of the group. Judging from the discontinuity in weight, this "normal" embryo of the pair is suffering a marked retardation and will probably be unable to hatch. This controversial pair was therefore eliminated, and the t value was calculated for the six remaining pairs. As shown by table 2, the t value for the six remaining pairs is 6.544 and highly significant.

The glutathione concentrations at 19 days are rather interesting. Although the mean values of the normal embryos have a slightly higher concentration than the lethal, the glutathione values are evidently converging at this age, whereas the weight values are diverging. The difference in glutathione values is not significant, but the difference in weight values is highly so. This weight—glutathione relationship at 19 days, though apparently inharmonious, can perhaps be explained. As Goss and Gregory ('35a) have shown, food greatly influences the glutathione concentration in new-born rabbits. Goss, Gregory, and Asmundson (unpublished data) have found that food greatly influences the glutathione concentration in young chickens a few days after hatching. If nutrition becomes a limiting factor at 19 days of incubation, this condition might be reflected in the glutathione value. The amount of contents of the yolk sac might throw some light on the food supply of normal and Cornish lethal embryos at this age; but unfortunately the yolk-sac contents were not weighed nor taken into account.

The ascorbic acid values are calculated for convenience as milligrams of glutathione per hundred grams of weight.* Here, again, it

*Earlier in our work the fraction of the tungstic acid filtrate, ascorbic acid, that was oxidized by 2, 6-dichlorophenolindophenol was calculated for convenience as glutathione, milligrams per cent. Any ascorbic acid value calculated as glutathione milligrams per cent in this or previous reports can be reduced to the proper ascorbic acid value by multiplying by the factor 0.29.

should be mentioned that ascorbic acid oxidizes readily and that special precautions, impractical for us to observe, are necessary to obtain absolute values. Since our analyses for an individual pair were made under similar environmental conditions, the ascorbic acid values may have considerable significance as to relative levels and trends during the incubation period, rather than absolute values. It may be observed (fig. 1, tables 1 and 2) that the ascorbic acid curves for normal and Cornish lethal are similar. There is a decline from the fourteenth to the nineteenth day. When the two groups are compared for a given day of incubation, none of the t values are significant.

The Creeper

The males used to produce embryos for studing the effect of the Creeper gene in the heterozygous state upon the glutathione concentra- in the embryo were supplied by Landauer. These animals were descended from the original Creeper stock crossed with White Leghorns. The embryos studied were produced by mating heterozygous Creeper males to homozygous normal White Leghorn females maintained at the poultry plant at Davis. This mating produced two classes of embryos—normal and heterozgous Creeper. All our comparative studies are between heterozygous Creeper and normal sibs. For the sake of brevity, heterozygous Creeper embryos are called simply *Creeper*. Length of tibia was the diagnostic character used for determining normal and Creeper genotypes. As Landauer ('34) has shown, this is a reliable index; and we found that the tibia length of the normal and Creeper genotypes did not overlap in the embryos studied.

Egg weights were taken into account in pairing the normal and Creeper embryos just as in pairing normal and Cornish lethals. The incubation periods studied were 14, 15, 16, 17, 18, and 19 days. All the Creeper data together with their normal sibs are summarized in

TABLE 3
SUMMARY OF ALL THE DATA OBTAINED FOR CREEPER AND NORMAL EMBRYOS

		Normal Means				Creeper Means			
Days of incubation	No. of pairs	Tibia length	Embryo wt. in grams	GSH mg. %	Ascorbic acid mg. %	Tibia length	Embryo wt. in grams	GSH mg. %	Ascorbic acid mg. %
14	45	1.83	9.0	42.7	26.4	1.42	8.75	42.1	24.8
15	7	2.06	11.6	43.3	25.6	1.61	11.32	44.1	27.1
16	9	2.4	14.7	38.5	19.0	1.85	14.1	37.9	18.0
17	9	2.7	17.7	35.3	16.0	2.01	17.7	38.0	15.9
18	7	2.8	20.6	36.1	16.1	2.1	19.5	35.1	15.0
19	22	3.1*	23.3	34.8	9.0	2.4*	21.9	35.5	8.5

*Includes tibia length of six pairs only.

table 3 and figure 2. The differences in tibia length between normal and Creeper embryos are significant for each incubation age studied. The curves for weight, ascorbic acid, and glutathione for the normal and Creeper groups (fig. 2) are quite similar and show the same trends for each character in question. None of the mean differences for weight, ascorbic acid, and glutathione are significant at any of the incubation ages studied.

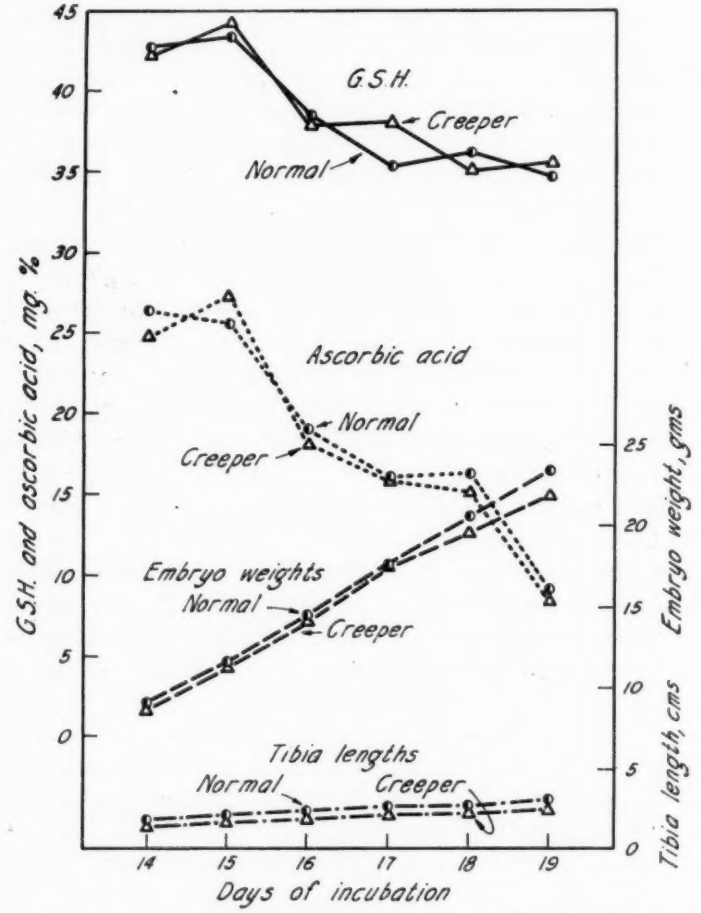

FIG. 2. Curves showing weights, tibia lengths, ascorbic acid, and glutathione for normal and Creeper embryos from 14 to 19 days of incubation. Data from table 3.

DISCUSSION

Landauer ('35b) has commented upon the similarity of morphological charateristics caused by the Creeper and the Cornish lethal genes. In the heterozygous state both genes reduce the length of the long bones of the extremities, the distal bones of the leg (tibia and tarsometatarsus) being most affected. Both genes cause the fibula to become overdeveloped. In the homozygous state both the Creeper and the Cornish lethal genes cause deformities of the head and extreme shortening of the extremities. On the other hand, the homozygous Creeper die early in the incubation period, whereas the homozygous Cornish lethal die late in the incubation period.

It should be emphasized that in testing the effect of each of these genes upon the various characters studied, normal sibs were used, in each case, for controls. The only consistent genetic difference between the mutant group in question and the sibs used as its control is, therefore, the mutation in question and its normal allele. The effect of the Cornish lethal gene was studied in the homozygous state, whereas the effect of the Creeper gene was studied in the heterozygous state. The homozygous Cornish lethal group is retarded in weight for all periods studied. The glutathione values of the Cornish lethal groups are consistently lower and in general agree with the lower weight values of the embryos.

The heterozygous Creeper embryos, on the other hand, show that the length of the extremities (based on tibia length) is significantly reduced; but no significant reduction in embryo weights occurs at any incubation age. The glutathione values of Creeper and controls are the same and are thus consistent with the total embryo weight values. Since the individual bones that make up the leg are shorter in the Creeper, one might expect the total weight of the leg of the Creeper to be less than the leg weight of normal sibs. These leg-weight differences between Creeper and normal embryos may be reflected in the glutathione values when legs alone are compared. A sufficiently sensitive microchemical method for the analysis of such small samples is not available.

When the glutathione curves of Cornish groups (normal and lethal) are compared with the Creeper groups (normal and Creeper), the curves of the Cornish and Creeper races show entirely different characteristics. In order that more races may be compared, the results of

our earlier analyses (Gregory, Asmundson, and Goss '36) for Barred Plymouth Rock and White Leghorn embryos are plotted with the Cornish and Creeper data. Glutathione values and embryo weight values of all the populations studied during the period of incubation from 14 to 19 days are included in figure 3. Evidently, the weight values for Rocks and Leghorns are slightly higher than for the two

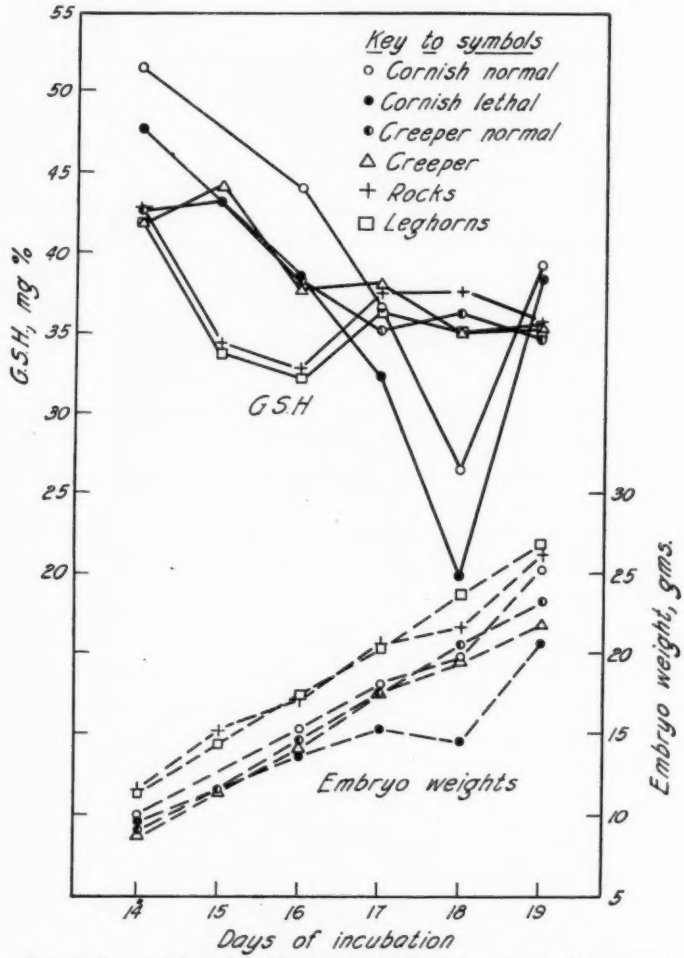

FIG. 3. Comparative glutathione and weight curves for Cornish, Cornish lethal, creeper, normal creeper, Rock and Leghorn embryos. Data from tables 1 and 3 of this report and from table 2, Gregory, Asmundson, and Goss ('36).

Creeper groups and for the normal Cornish group. All of these five weight curves, however, have about the same slope. The weight values of the Cornish lethal embryos are lower for most of the incubation periods, and this curve appears to have a different slope when compared with the other five weight curves. When the glutathione curves of all six populations are compared, three characteristic types of curves are recognizable; namely, Cornish, Creeper, and Rock-Leghorn.

Because of the striking parallelism between the two Cornish curves (normal and lethal), they are considered as one type even though the values of the Cornish lethal embryos are lower at each point. Similarly, the Rocks and Leghorns constitute one type of curve even though the glutathione values of the Rocks are slightly but consistently higher for each incubation period. The differences in types of glutathione curves for these populations appear more striking than the differences in curves for weight. Our data show that glutathione values are more variable than weight values. The differences within the groups constituting these three general types of curves have been discussed earlier in this report and in a previous paper.

Environmental factors do influence the type of glutathione curve to a certain extent. In analyzing three series of Barred Plymouth Rock and White Leghorn embryos from 10 to 19 days of incubation Gregory, Asmundson, and Goss ('36) (fig's. 3 and 5) found some variability between embryos of the three series hatched from different batches of eggs from the same parents. The characteristic patterns of the glutathione curves of series 2 and 3 are quite similar for each series of Rocks and each series of Leghorns. Series 1, when compared with series 2 and 3, appears obviously different. In it the peak in concentration occurs on the thirteenth day, whereas in series 2 and 3 the peak occurs at 14 days. When the curves of the three series of Rocks and Leghorns are considered, series 1 seems to have been pushed forward about one day. Evidently whatever factor (or factors) influenced the glutathione concentration in series 1 operated also in both Rock and Leghorn embryos. This variability between series 1 on the one hand and series 2 and 3 on the other was undoubtedly caused by uncontrolled environmental conditions and not by differences in genetic constitution.

In considering these three characteristic types of glutathione curves of Cornish, Creeper, and Rock-Leghorn, one should remember, however, that in the designs of the original experiments Rocks were

paired against Leghorns, Creeper against normal sibs, and Cornish lethal against normal Cornish sibs, so that in each specific comparison environmental conditions were carefully controlled and, as a result, any differences between the groups compared might be attributed to differences in genetic constitution of the races in question.

Comparisons are made without regard to the designs of the original experiments, in figure 3 of this report. Even though characteristic types of glutathione curves are recognizable, one encounters certain difficulties in deciding whether or not the characteristic types of curves are caused by differences in genetic constitution or by uncontrolled environmental factors such as differences in egg weights, differences in incubation conditions, or differences in conditions in the laboratory at the time of analysis. Temperature in the laboratory may vary as much as 25° F. between summer and winter, and this wide range may influence the glutathione value to a slight extent. All these environmental variables were well controlled in the original paired experiments. Critical data concerning the relations of genetic constitution and environmental factors to the characteristic types of glutathione curves in question may be obtained by analyzing Cornish, Creeper, and Rock (or Leghorn) embryos simultaneously, taking into account egg weights and controlling all environmental factors possible, as in the paired experiments. If the pattern of the glutathione curve is determined largely by genetic constitution, the pattern of the curve may have considerable significance from a developmental standpoint in analyses of differential racial development where genetic constitution for adult size, rate of growth, or heterosis is involved, even though embryo weights during the period in question are about the same.

SUMMARY

Two qualitative genes, Cornish lethal and Creeper, both of which are lethal in the homozygous state and retard the rate of growth, were investigated from 14 to 19 days of incubation. The object was to determine whether or not qualitative genes that retard the rate of growth will simultaneously influence the amount of glutathione in the body tissue. In each experiment the mutant embryos were paired with normal sibs for control. Original egg weights were taken into account in pairing; each embryo of a pair was incubated in the same incubator

at the same time; and the chemical determinations were made under similar environmental conditions.

Homozygous Cornish lethal embryos are definitely retarded in weight throughout the period. The glutathione curves for normal Cornish and Cornish lethal embryos from the fourteenth to the nineteenth day of incubation are quite similar even though the glutathione values of the Cornish lethal are consistantly lower than for normal embryos and the differences are significant for all days except the nineteenth. Differences in ascorbic acid values between normal Cornish and Cornish lethal embryos are not significant.

Heterozygous Creeper embryos are not retarded in weight. Glutathione values of heterozygous Creeper and normal sibs are approximately the same and apparently agree with the weight values of the embryos. There is no difference in ascorbic acid between heterozygous Creeper and normal sibs.

Characteristic types of glutathione curves were observed for the different populations studied. Their possible significance is discussed.

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THE PRENATAL GROWTH OF THE CAT

VIII. THE WEIGHTS OF THE KIDNEYS, BLADDER, GONADS AND UTERUS, WITH WEIGHTS OF THE ADULT ORGANS

By

HOMER B. LATIMER

Department of Anatomy

University of Kansas

(*Received April 29, 1939*)

The kidneys seem to have received more attention quantitatively, in both gross and microscopic structure than the other parts of the urogenital system. Quantitative data on the other organs are very meager and it is to partially fill this need that this paper is presented.

The postnatal growth of the cat kidneys have been reported by Hall and MacGregor ('37) and the weights of the adult cat kidneys are given by Roaf ('13). Markie and Hinsey ('35) give the uterus weight for two cats. Denzer ('38) has gathered much of the available data on kidney weights in different species of animals, together with data on the histological quantitative studies. Hopkins and Biely ('35) give the weights of the kidneys in the chicken and Donaldson ('24) gives data for all of the organs reported in this paper, for the albino rat. Rowlands and Brambell ('33) have studied the postnatal growth of the testes in the albino mouse and Schulz ('38) has reported the weights of the testes in several species of primates. The weights and dimensions of the human kidney and a few weights of the three other organs are given by Peter ('27) for the postnatal period, and a recent study of the adult human kidney weights has been made by Wald ('37).

MATERIAL AND METHODS

The 229 fetal and 35 newborn cats used in this study have been described in the first paper of this series (Latimer and Aikman, '31) and the 104 adult cats have been described in a later paper (Latimer, '36). In removing the kidneys, the capsule was removed and the ureter and blood vessels cut close to the hilus of the kidney. The ureters and the urethra were cut close to the bladder and it was emptied of all urine. The gonads were removed free of all mesenteries and vessels and the epididymis was removed from the testes. The uterus weight does not include the vagina and all mesenteries and vessels were cut close to the uterus as well as the other organs. All of these organs were removed as

rapidly as possible upon opening the body cavity and they were kept in a moist chamber together with the other organs until removed and weighed in glass stoppered weighing bottles. All weights were made on a chemical balance sensitive to 0.1 milligram. The same methods were used for all of the cats and the dissecting and weighing was done by the same person. The fetal and newborn cats had been preserved in formalin but the adult cats were weighed in the fresh condition.

The method of deriving the fomulae for the curves to be presented later has been described (Latimer, '33) and will not be repeated here. The formulae used in computing the data for the tables are given by Dunn ('29).

I wish to most gratefully acknowledge the assistance of Miss Edna Mae McConnell who assisted with the compiling of the data for the adult cats.

KIDNEYS

The prenatal growth of the kidneys is shown in figure 1. The curve

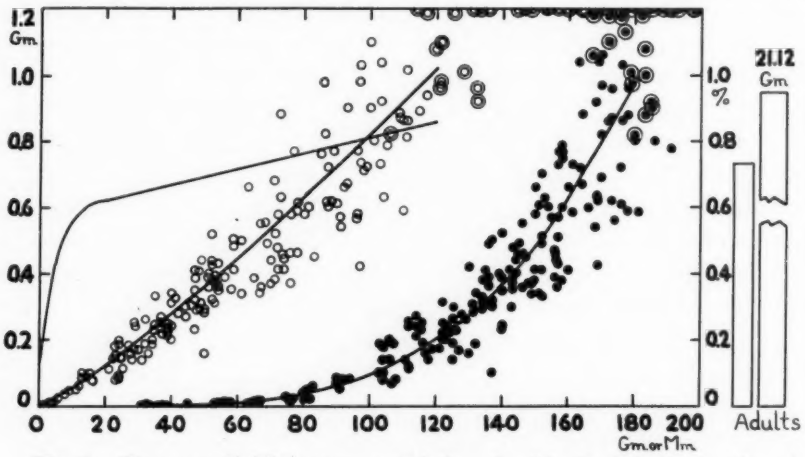

Fig. 1. The curve slightly concave superiorly and with the individual pairs of kidney weights shown as circles gives the growth of the kidneys plotted on body weight. The double circles represent the weights of the two kidneys from the newborn cats. The curve with the kidney weights represented by dots gives the growth in weight of the two kidneys plotted on body length. The dots enclosed by circles give the kidney weights for the newborn cats. Formulae for both of these curves are given in the text. The lighter line without any cases represents the weight of the two kidneys as a percentage of the total body weight, with the percentage values given at the right. The shorter column represents the average percentage weight and the taller broken column represents the average weight in grams of the two kidneys in the adult male cats.

slightly concave superiorly and with the weights of the pairs of kidneys shown as circles gives the increase in weight of the kidneys plotted on body weight. The newborn kidneys are heavier than the late fetal kidneys and are shown along the top of the chart as double circles. Several of these kidneys weighed over two grams and one pair weighed 2.50 grams. The formula from which this curve was drawn is,

$Y = 0.00326(X^{1.2}) - 0.0007$, from 0.6 to 120 grams of body weight, with Y representing the weight of the two kidneys in grams and X, the body weight also in grams.

The curve concave superiorly and with the weights of the pairs of kidneys shown as dots, gives the kidney weights plotted on body length. The dots enclosed by circles show the weights of the newborn kidneys. The formula used in drawing this curve is,

$Y = \frac{(0\,1X)^4}{106700} - 0.00164$, from 40 to 180 mm. of body length. In this formula Y represents the weight in grams of the pairs of kidneys, and X, the body length measured in millimeters. To determine the accuracy of the fit of these formulae, the percentage deviations of the calculated from the observed values for each ten grams, or ten millimeters, increase in body weight or body length were found, and the highest average percentage deviation of all of the formulae for the urogenital system was found for the kidney weight on body length, or an average of 11.49 per cent. The fit of the curve on body weight is better for the average deviation is but 5.24 per cent, which is next to the lowest.

The lighter line in this figure, without any cases shown, represents the weights of the two kidneys as percentages of the body weight and plotted on body weight. This curve shows that the kidneys increase much more rapidly at first than does the body weight and then after about fifteen grams of body weight they increase more slowly. This line if carried on for the newborn kidneys is too low but the percentage weights are not as far above the line as are the absolute weights of the kidneys. The cases are not shown with the percentage curves in any of these figures, but only the smoothed curve connecting the averages for each ten grams increase in body weight and taken from the field graph.

The shorter column at the right gives the percentage weight and the taller column, the weight in grams of the two adult male kidneys. The weights in grams and the percentage weights of both male and female

pairs of kidneys are given in table 1. The last column of table 1 gives the significant ratio or the difference between the weights of the male and female kidneys divided by their probable error. The male kidneys are significantly heavier. The ratio for the percentage weights is 2.85 which would indicate that the male kidneys probably form a larger percentage of the body weight.

The level of the two kidneys was determined for 232 of the fetal series and for all of the adult cats. In 74.3 per cent of the fetal and new born cats the right kidney was more cephalad, varying from just appreciably higher, to one half the length of the kidney above the upper border of the left kidney. In all of the adult males, the right kidney varied from slightly higher to a distance equal to half the length of the

TABLE 1
WEIGHTS IN GRAMS AND PERCENTAGE WEIGHTS OF THE ORGANS

	Fifty-two males		Fifty-two females		Difference
	Average weight	Coefficient of variation	Average weight	Coefficient of variation	P. E. diff.
	A. WEIGHTS OF THE ORGANS IN GRAMS				
Kidneys	21.118 ± 0.788	39.91 ± 3.03	16.890 ± 0.430	27.19 ± 1.93	4.71
Bladder	2.919 ± 0.708	25.94 ± 1.83	2.489 ± 0.070	30.07 ± 2.16	4.31
Gonads	2.016 ± 0.105	55.63 ± 4.69	0.220 ± 0.0085	40.87 ± 3.17	—
Uterus	— —	— —	1.525 ± 0.065	43.32 ± 3.53	—
	B. PERCENTAGE OF BODY WEIGHT				
Kidneys	0.737 ± 0.015	21.73 ± 1.50	0.687 ± 0.0089	13.82 ± 0.93	2.85
Bladder	0.103 ± 0.0018	19.06 ± 1.31	0.103 ± 0.0024	25.15 ± 1.77	0.10
Gonads	0.068 ± 0.0025	39.13 ± 2.96	0.0090 ± 0.00022	26.11 ± 1.84	—
Uterus	— —	— —	0.064 ± 0.0023	36.36 ± 2.84	—

kidney and in the adult females 88.5 per cent had the right kidney at a higher level. Two of the females had the left kidney higher and in four cats they were at the same level.

In 137 of the fetal series and in one half of the adult cats the right and left kidneys were weighed separately. In the fetal and new born cats the right kidney was heavier 71 times, the left 65 times and of the same weight once. In the adult males the right kidney was heavier 57.7 per cent of the time and in the adult females, 55.8 per cent. Furthermore, the significant ratio, or the difference between the average weight of the right and the left adult kidneys divided by the probable error of this difference was found to be 0.04 for the male kidneys and 0.23 for the female kidneys, or additional evidence of no bilateral asymmetry. Hall and MacGregor ('37) also find that there

is no significant difference in the weights of the right and left kidneys in the cat.

The percentage weight of the kidneys in my female cats comes within the range given by Hall and MacGregor ('37) but in my males the kidneys are a little lighter. Roaf's data ('13) give percentage weights of the male and female kidneys of 1.18 and 0.91 per cent respectively, which are higher than either of the other groups. The percentage weight of the kidneys of the adult female cat (0.69%) is very similar to the percentage of 0.64 per cent reported for the single comb white Leghorn pullet by Hopkins and Biely ('35). The figures given by Donaldson ('24) for the larger adult rats, and by Liesenfield, Dahmen and Junkersdorf ('27) for the dog fetus are greater, while the human kidney according to Scammon ('33) forms a smaller percentage of the body weight. Wald ('37) finds the human kidney much less variable than are these cat kidneys. The average of the coefficients of variation for his groups, omitting the last ten year group, are 18.7 per cent for the males and 20.9 per cent for the females. These are much lower than the coefficients given in table 1 and he finds the male kidneys less variable while in the cat the female kidneys are a little over 30 per cent less variable than the male kidneys.

Roaf ('13) gives the body weight and the kidney weight of 26 male and 22 female cats, and from these weights the statistical constants have been determined so that they might be compared with my series. The average body weight of his male cats is four per cent heavier but the kidney weights average over sixty-two per cent heavier than in my series. The females of his series average four per cent lighter in body weight, but twenty-five per cent heavier in average kidney weight, or the kidneys of Roaf's cats are relatively heavier than in my series. The siginficant ratios for the body weights and for the kidney weights of Roaf's male and female cats were found by using his data to be 7.01 for the body weights and 8.45 for the kidney weights, or his cats show a slightly greater sex difference than is shown in table 1 for my series. Comparing the coefficients of variation of body weights of this series (Latimer, '36) or 25 per cent for the males and 21 per cent for the females, we find respectively 19 and 13 per cent, or Roaf's cats are much more uniform in body weight. Likewise the kidneys are more uniform in weight for the coefficients of his series are 30.7 for the males and 22.3 for the females.

Hall and MacGregor ('37) have studied the kidney weight in one hundred cats from time of birth to maturity. Using their formula for determining the kidney weight from body weight, the percentage differences between the observed and the calculated values have been determined for both male and female adult cats. For my adult female cats the formula using the 0.67 power of the body weight gives better results, but for my adult males and for the newborn cats, both males and females, the formula using the 0.71 power of the body weight gives better results. Both of their formulae fit my specimens better than their specimens.

To see whether better formulae could be developed for my cats, the weights of the kidneys of the male and female adult cats were plotted on body weight and formulae developed for these in the same manner as for the fetal cats. The formula for the male kidneys is:

$Y = 0.0089X - 3.4$, from 1700 to 4600 grams of body weight.

The formula giving the weight of the female kidneys is:

$Y = 0.0069X - 0.205$, from 1500 to 4000 grams of body weight.

In these formulae Y represents the weight of the two kidneys in grams and X, the body weight. These formulae fit my adult cats, especially the females, better than either of the formulae of Hall and MacGregor. The average percentage deviation for the males is 7.21 per cent and for the females 1.15 per cent. Neither of these formulae fit the newborn kidney weights however.

In general, the percentage weight of the two kidneys increases in the fetal period with the average for the adult males slightly less than that in the late fetal period. For the adult cats in my series and in the series studied by Hall and MacGregor the percentage weights of the male kidneys tend to increase in the heavier cats and to decrease very slightly in the heavier adult female cats. The weights of the two kidneys in the newborn cats are not predicted with any accuracy by either of the formulae for the fetal cats but the second formula given by Hall and MacGregor ($K.\ W. = 9.825\ (B.\ W.)^{0.71} - 1.09$) predicts the kidney weight in both male and female newborn and also in my adult female cats and with a little less accuracy the adult males. Thus formulae are now available for predicting kidney weight from either body weight or body length during the fetal period and from body weight from birth to maturity.

BLADDER

The urinary bladders of the fetal cats were not weighed unfortunately and so data on only the adult bladders are available. Table 1 gives the average weights of the male and female bladders and the last column shows that the male bladder is significantly heavier. Panel B. of table 1 shows that the average percentage weights are the same in both sexes but with a significant difference of 0.10 in favor of the male bladder. This is due to the fact that more decimal places were used in making the determinations and these rounded to three decimal places for this table. The five place percentage weights of the male and female bladders are respectively, 0.10346 and 0.10317.

The percentage weights of the urinary bladders are given but to provide a more accurate method of determining the weight of the bladder in adult cats of various sizes, formulae were developed as for the kidneys. The formulae for the male are;

$Y = 0.00124X - 0.541$, from 1700 to 3300 grams of body weight and,

$Y = 0.00044X + 2.188$, from 3300 to 4400 grams of body weight.

Y represents the weight of the two kidneys in grams and X, the body weight. The average percentage deviation for these two formulae are respectively, 0.36 and 3.78 per cent. The formula for the weight of the female bladder is:

$Y = 0.00082X + 0.6$, from 1500 to 2800 grams of body weight.

From 2800 to 3400 grams of body weight the bladder averages 2.896 grams, or the curve is here a horizontal line. The average percentage deviation of the above formula is 1.88 per cent.

The average percentage weigth of the bladder in both sexes is given in table 1 as 0.103 per cent. To get some idea of the changes in the relative weights of the bladders in all of the adult cats, the percentages were plotted on the body weights. In the males there was no appreciable change, for the line connecting the averages for the various groups formed practically a horizontal line. In the females, the line representing the percentage weight of the bladder started at about 0.12 per cent in the smallest adult females and decreased regularly to about 0.08 per cent in the largest females.

In general we may say that the urinary bladder in the male cat is less variable than in the female and it does not change in its percentage of body weight throughout the range of gross body weight. In the adult

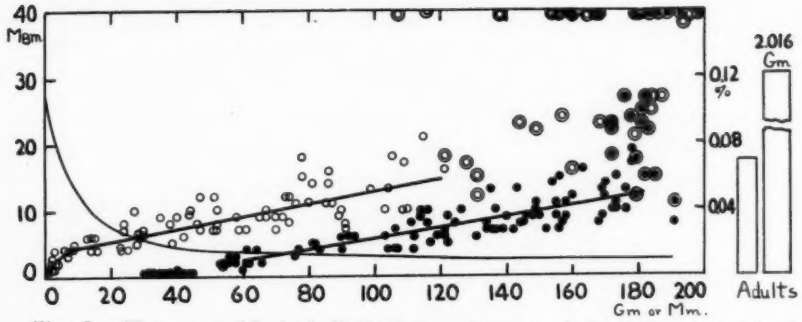

Fig. 2. The curve with the individual cases shown as circles, gives the ponderal increase of the two testes plotted on body weight. The double circles show the weights of the testes in the newborn cats. The straight line curve with the cases shown as dots represents the growth of the testes on body length. The dots enclosed by circles give the weights of the testes in the newborn cats. Formulae for both curves are given in the text. The lighter line without any cases represents the percentage weights of the testes. The percentage values are given at the right. The shorter column represents the average percentage weight of the testes and the taller broken column, the absolute average weight of the adult testes.

female, the bladder is more variable in both absolute and in percentage weight and it forms a relatively larger percentage of the body weight in the smaller adult females.

TESTES

The increase in weight of the testes in the fetal period plotted on body weight is shown in the curve to the left in figure 2 and with the weights of the pairs of testes shown as circles for the fetal cats and as double circles for the newborn cats. The straight line curve with the cases shown as dots for the fetal cats and as dots enclosed by a circle for the newborn represents the growth of the testes plotted on body length. The formulae for the curve plotted on body weight are as follows:

$Y = X^{0.5} + 0.15X + 0.301$, from 0.6 to 10 grams of body weight,

$Y = 0.094\ (X\text{-}10) + 4.963$, from 10 to 120 grams of body weight

In the above formulae Y represents the weight of the two testes in milligrams and X the body weigth in grams. The formula for the second curve is:

$Y = 0.08X - 2.28$, from 60 to 180 millimeters of body length.

In this formula Y represents the weight of the two testes in milligrams and X, the body length measured in millimeters. Most of the testes of the newborn specimens are much heavier than the testes of the late fetal cats and these are shown along the upper border of the

figure. All but two of these shown along the top of the figure weighed over 100 mgm. and the heaviest weighed 142 milligrams. The average percentage deviation of the calculated values for the two formulae based on body weight is 8.55 per cent, and for the formula for body length, 8.50 per cent.

The lighter line without any cases and with the percentages shown at the right represents the testes as a percentage of the body weight. The percentage weight of the testes averages about 0.11 per cent at 0.6 gram of body weight and drops rapidly at first and then more slowly to about 0.013 per cent. The cases were rather closely grouped about the line in the field graph (not shown) and there was no transition from the late fetal to the percentage of the newborn as is shown for the absolute weights in this figure.

The first column at the right represents the percentage of the adult testes, or 0.068 per cent of the body weight (table 1) and the second or broken column represents the absolute weight of the adult testes or 2.016 grams. The coefficient of variation of both the absolute and the relative weights of the testes are given in table 1 and both are very high, that for the absolute weight is the highest coefficient given in this table. The weights of the testes of the adults were plotted on the body weight and a line drawn through the averages for each group. The formula for this line, which gives a better estimate of the testes weight for the various body weights than the average given in table 1, is as follows:

$Y = 0.00135X - 1.686$, from 1700 to 4600 grams of body weight.

Y represents the weight of the two testes in milligrams and X the body weight in grams. There is a good deal of variation in the scatter of the cases on the field graph (not shown) and the average deviation of the calculated values from the observed is 9.58 per cent. The percentage weights of the testes of the adult cats were plotted on body weights as were the absolute weights, and the averages connected by a straight line which increases from about 0.04 per cent at 1700 grams of body weight to about 0.1 per cent at 4400 grams of body weight.

The right and left testes of 93 of the fetal cats and 22 of the adult cats were weighed separately to see if there might be any asymmetry in their weights. The results do not show any definite asymmetry for in the fetal cats the right testes are heavier in 50.7 per cent of the cases and in the adult cats the left testes are heavier in 54 per cent. The

significant ratio of the right and left adult testes was found to be but 0.12.

In the smallest specimens the gonads were not distinguishable in gross dissection as either testes or ovaries and they were always found in the embryonic position, or high in the posterior body cavity. By about three grams of body weight the testes were becoming more spherical while the ovaries were assuming a fusiform shape but both were still in the first position. In the specimens of from 20-30 grams of body weight a few of the testes had started to migrate but many were still in the original position. From 40-50 grams of body weight about half of all the testes were close to the inner inguinal ring and the rest were nearer the inguinal region than the original position. At 70-80 grams of body weight nearly all of the testes were close to the abdominal wall and the inguinal ring. At 90 grams of body weight there was one specimen with both testes just entering the inguinal canal and in both testes the epididymis was in a cephalad position, and this position of the epididymis was observed in several other specimens later on. Between 100 and 110 grams of body weight the testes were either close to the abdominal ring or in the inguinal canal and in only one late fetus was one testes found in the scrotum. In the newborn specimens the testes were usually in the scrotum or with one testes only in the canal.

In general the migration of the testes in the cat is similar to that of other mammals. The left testes seem to lead slightly in this migration. They do not enter the scrotum until just about the time of birth. The small number of glands found between the original position high in the abdominal cavity and the abdominal inguinal ring and also within the inguinal canal, would seem to indicate a more rapid rate of migration through these two regions. In both the fetal and in the adult cat there is no significant difference in the weight of the left and right testes. The testes are exceedingly variable in weight, and are relatively heavier in the early than in the late fetus.

OVARIES

The increase in weight of the two ovaries plotted on body weight is shown as the straight line beginning at the left in figure 3. The weights of the pairs of ovaries are shown as circles for the fetal cats and as double circles for the newborn cats. This curve was drawn from the formula:

$Y = 0.15X + 0.73$, from 0.6 to 120 grams of body weight.

In this formula Y represents the weight of the two ovaries in milligrams and X, the body weight in grams.

The curve concave superiorly and starting farther to the right represents the growth of the two ovaries plotted on body length. The weights of the two ovaries are represented as dots for the fetal cats and as dots enclosed by circles for the newborns. The formulae for this curve are as follows:

$Y = 0.037X - 1.02$, from 60 to 90 mm., and

$Y = \frac{(0.1X)^3}{315}$ from 90 to 180 mm. of body length.

In the above formulae Y represents the weight of the two ovaries in

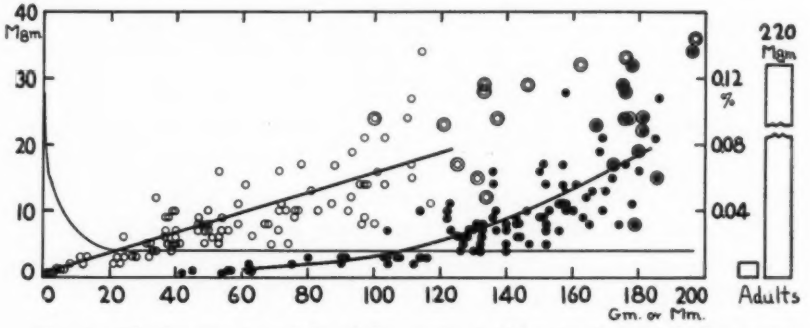

Fig. 3. The increase in weight of the ovaries is shown in the straight line curve, with the individual pairs of ovaries shown as dots, plotted on body weight. The double circles represent the weights of the newborn ovaries. The curve concave superiorly and with the cases shown as dots gives the increase in the weights of the ovaries plotted on body length. The dots enclosed by circles represent the ovarian weights in the newborn cats. Formulae for both curves are given in the text. The lighter line without any cases represents the ovarian weights as percentages of the body weight. The percentage values are given at the right. The shorter column represents the percentage weights of the ovaries in the adult cats and the taller broken column represents the average weight of the adult ovaries.

milligrams and X the body length in millimeters. The average percentage deviation of the calculated from the observed values for each ten grams or ten millimeters increase is 9.24 per cent for the curve on body weight and 7.17 per cent for the curve based on body length. Unlike the kidneys, the testes and the uterus, the weights of the newborn ovaries are fairly well in line with the growth of the fetal ovaries.

The lighter line in this figure without any cases shown represents the weight of the ovaries as percentages of the body weight. The percentage values are shown at the right of the figure and it will be

seen that the ovaries decrease rapidly at first and then from 25 to 200 grams of body weight they form 0.016 per cent of the body weight. The shorter column at the right represents the percentage weight of the ovaries in the adult cats, or 0.009 per cent of the body weight (table 1). The taller broken column represents the absolute weight of the two ovaries.

The average weight, both absolute and percentage, of the ovaries given in table 1 shows that the ovaries are smaller than the testes and also they are less variable. The weights of the ovaries of the adult cats were plotted on body weight and regression lines were drawn through the averages for each 500 grams increase in body weight as for the preceding organs and the best fit was obtained by breaking the line into two straight lines drawn from the following formulae;

$Y = 0.000012X + 0.163$, from 1500 to 2600 grams and,

$Y = 0.000143X - 0.178$, from 2600 to 3400 grams of body weight. The average percentage deviation of the calculated from the observed values for each 500 grams increase in body weight for these formulae is but 0.94 per cent. The percentage weights of the pairs of ovaries were likewise plotted on body weight and a straight line drawn through their averages for each 500 gram group and this line started a little above 0.01 per cent and decreased to 0.006 per cent of the body weight.

The right and left ovaries were weighed separately in 101 fetal and 22 adult cats and while they seem to indicate a heavier ovary on the right side more frequently the percentages are not sufficiently large in the fetal cats to indicate asymmetry. In the fetal cats the right ovary was heavier in 44.56 per cent; the left ovary was heavier in 38.61 per cent and both were of the same weight in 16.83 per cent of the cases. In the adult cats the results are more conclusive, except for the small number of cases, for the right was heavier in 81.81 per cent, the left in 13.64 per cent and both were of the same weight in 4.55 per cent of the cases. The significant ratio for the average weights of the right and left adult ovaries is higher than any of the similar ratios or 1.60 It is still not great enough to be significant.

To summarize, the ovaries are relatively and absolutely lighter than the male gonads and also less variable in weight. In the adult cats, the percentage weights of the testes increase somewhat with increase in body weight, but the ovaries decrease in percentage weight with

increase of body weight. There is no significant difference in the weight of the two ovaries.

UTERUS

The increase in weight of the uterus plotted on body weight is shown in figure 4 as the curve starting at the left and with the weights of the individual uteri shown as circles for the fetal cats and as double circles for the newborn kittens. This curve was drawn from the formula,

$Y = 0.0081(X^{1.9}) + 4.42$, from 10 to 120 grams of body weight.

In this formula Y represents the weight of the uterus in milligrams and X, the body weight in grams.

The same weights plotted on body length are shown in the curve starting farther to the right and with the weights of the individual uteri shown as dots for the fetal cats and as dots enclosed by circles for the newborn cats. The formula for this curve is:

$$Y = \frac{(0.1X)^{4.1}}{1572} - 0.222x + 19.98, \text{ from 90 to 180 mm. of body length.}$$

In this formula Y represents the weight of the uterus in milligrams and

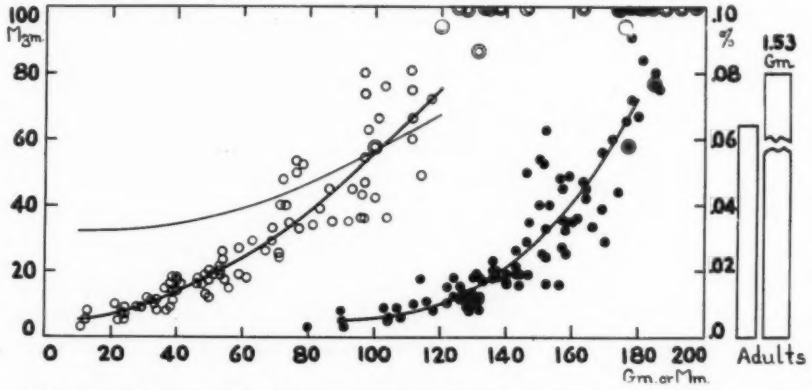

Fig. 4. The curve with the cases shown as circles represents the growth of the uterus plotted on body weight. The double circles represent the uterus weight in the newborn cats. The curve with the cases shown as dots gives the growth of the uterus plotted on body length. The dots enclosed by circles represent the weights of the uteri in the newborn cats. Formulae for both curves are given in the text. The lighter line without any cases represents the uterus as a percentage of the body weight. The percentage values are given at the right. The shorter column at the right gives the average percentage uterine weight in the adult and the taller broken column gives the absolute average adult weight of the uterus.

X, the body length in millimeters. These two formulae do not give the weights of the uteri of the newborn cats. The average percentage

deviation of the calculated weights of the uteri from the observed weights plotted on body weight is 3.30 per cent or the best fitted of any of the formulae for the fetal organs. The average deviation of the calculated weights of the uteri for the body length is 8.97 per cent. The uterus was not weighed in the smaller specimens and so these curves begin at either ten grams of body weight or ninty millimeters of body length.

The lighter curve without any cases represents the percentage weight of the uterus. The values for this curve are given at the right and increase from about 0.032 per cent to about 0.064 per cent. The shorter column at the right represents the percentage weight of the uterus in the adult cat or 0.064 per cent (table 1). The taller broken column represents the average weight of the uterus in the adult cat. Johnson ('33) states that the human uterus is relatively larger in the fetus and newborn than in the adult, but this is not found in the cat. So far as is known there are no data on the postnatal changes in the cat uterus. The two cat uteri reported by Markie and Hinsey ('35) fall within the limits of variation of the uteri of this group of cats but they average 13 per cent lighter than the average for my group of cats.

The cases in figure 4 scatter rather widely about the two curves and the coefficients of variation given in table 1 show that the absolute weight and the percentage weight of the uterus is the most variable of any of the adult female organs and second only to the testes. The menstrual cycle may account to some extent for the variability in the weights of the adult uterus and ovary. This will not explain the variability of the testes however.

The weights of the nonpregnant adult uteri plotted on body weight are best represented by a straight line increasing with the increase in body weight and drawn from the formula,

$Y = 0.00104X - 0.84$, from 1500 to 3000 grams of body weight.

In this formula Y represents the weight of the uterus in grams and X, the body weight in grams. The average deviation of the calculated from the observed averages is 3.44 per cent. The percentages of the uterus weight on body weight of the adult cats were also plotted on body weight and the line through the average for each 500 grams increase in body weight is nearly a horizontal line, increasing from 0.055 to 0.07 per cent of the body weight.

The uterus was not weighed in the smallest specimens but in the later

part of the fetal period it increases very rapidly. In the adult it increases in proportion to the increase in body weight. It is the most variable of the adult female organs studied and second only to the testes in coefficient of variability.

CORRELATIONS

The correlation between body weight and body length and the four organs of the urogenital system, and also the correlations between the four organs are given in table 2. These correlations are only for the adult cats.

All of the correlations are positive although some are so low that they

TABLE 2
CORRELATIONS

	Males	Females
Body weight, and, kidney weight	+0.837 ± 0.028	+0.868 ± 0.023
bladder weight	+0.788 ± 0.035	+0.503 ± 0.070
gonads weight	+0.828 ± 0.029	+0.687 ± 0.050
uterus weight	— — — — —	+0.506 ± 0.070
Body length and, kidney weight	+0.593 ± 0.061	+0.734 ± 0.043
bladder weight	+0.851 ± 0.026	+0.448 ± 0.075
gonads weight	+0.737 ± 0.043	+0.149 ± 0.097
uterus weight	— — — — —	+0.607 ± 0.062
Kidney weight and, bladder weight	+0.863 ± 0.024	+0.551 ± 0.065
gonads weight	+0.747 ± 0.041	+0.666 ± 0.053
uterus weight	— — — — —	+0.599 ± 0.060
Bladder weight and, gonads weight	+0.863 ± 0.024	+0.334 ± 0.084
uterus weight	— — — — —	+0.624 ± 0.060
Ovaries weight and uterus weight	— — — — —	+0.114 ± 0.098

are not significant. All of the correlations are higher in the male cats with the exception of the kidney weights correlated with both body weight and body length. The average of the nine correlations in the male is + 0.790 and for the fourteen correlations in the females, + 0.528. All of the organs are better correlated with body weight except the male bladder and the uterus. The highest correlation is found between the body weight and the weight of the kidneys, and the lowest between ovaries and the uterus. The correlations with the testes are all fairly high, while the correlations with the ovaries are all low and not significant except those with the body weight and with the weight of the kidneys. The weights of the ovaries were plotted on the weights of the uterus of the adult cats with the resulting line nearly horizontal for the weights of the ovaries. This, together with the changes in the per-

centages of these adult organs given above, would explain the low correlation between these two organs.

The body weights and the weights of the kidneys of the cats used by Roaf ('13) were given in his publication and so correlations have been determined to see how these would compare with the correlations in my series. The correlation between body weight and kidney weight in the male cats is $+ 0.784 \pm 0.051$ which is less than the similar correlation in my series, but the correlation for the females is slightly higher or $+ 0.890 \pm 0.030$.

In general the body weight is a better indication of the weights of these organs of the urogenital system than is the body length, and the correlations are higher in the males than in the females. It has been shown in table 1 that the weights of these organs and in earlier papers (Latimer, '36 and '38) that the weights and linear dimensions of the cat and the weights of the central nervous system are more variable in the male cats, and in all of these the correlations are higher in the males which must indicate that although the males vary in body size and in size of these organs a great deal, they keep the same relative proportions to a greater degree than do the females.

CHANGES IN RELATIVE PROPORTIONS

The changes in the relative proportions of the four organs with reference to body weight and body length and also with reference to each other are shown in table 3.

The kidneys increase from their minimum percentage of the body weight in the early fetus to their maximum at birth and then decrease slightly in the adult male and a little more in the female. Their minimum with reference to body length is found in the early fetus. They about double this percentage by the end of the fetal period and increase it over 1700 times in the adult male and over 1400 times in the adult female. The ratios show that the kidney of the adult male cat is over seven times as heavy as the bladder and a little less than seven times in the female. With reference to the testes, the kidneys are at their minimum in the early fetus and at their maximum in the late fetus. The kidneys increase more rapidly than the ovaries in both the prenatal and postnatal periods. The kidneys and uterus maintain approximately the same relative weights throughout the entire growth period, with a slight increase in the kidneys in the late fetus.

The bladder is slightly heavier with reference to the body length in

the female cat, and it is once and a half heavier than the uterus in the adult. The bladder is about once and a half heavier than the testes but over eleven times heavier than the ovaries.

The gonads are of equal weight at the beginning of the fetal period and then in the late fetus the testes form 82 per cent of the weight of the ovaries but in the adult they are over nine times as heavy. The uterus increases its ratio to the weight of the ovaries in the fetal period and in the adult it is still heavier.

TABLE 3

CHANGES IN THE RELATIVE PROPORTIONS OF THE PARTS OF THE UROGENITAL SYSTEM

		Early fetus	Late fetus	Adults	
				Male	Female
Percentage of body weight:					
	Kidneys	0.18	0.86	0.74	0.69
	Testes	0.108	0.013	0.068	—
	Ovaries	0.108[1]	0.016	—	0.009
	Uterus	0.032[2]	0.064	—	0.064
Percentage of body length:					
	Kidneys	0.0023	0.0050	4.05	3.32
	Bladder	—	—	0.42	0.49
	Testes	0.0018	0.0067	0.39	—
	Ovaries	0.0018	0.0096	—	0.043
	Uterus	0.0058[3]	0.0384	—	0.30
Ratios:					
	Kidneys / Bladder	—	—	7.24	6.79
	Kidneys / Testes	1.70	66.55	10.48	—
	Kidneys / Ovaries	1.71	53.37	—	76.77
	Kidneys / Uterus	10.08[2]	13.28	—	11.08
	Bladder / Gonad	—	—	1.45	11.31
	Bladder / Uterus	—	—	—	1.63
	Testes / Ovaries	1.00	0.82	9.16	9.16
	Uterus / Ovaries	2.27[2]	4.09	—	6.93

[1] Observed weight used to determine percentage

[2] at 10 grams of body weight

[3] at 90 mm. of body length

In general, the kidneys and uterus increase in relative weight in the fetal period. The kidneys are relatively a little lighter in the adult, and the adult uterus is practically the same relative weight as in the late fetus. The gonads are both at their maximum relative weight in the early fetus and their absolute weights are the same at this time. Both decrease in the fetal period with the ovaries forming a slightly larger percentage of the body weight in the late fetus. The adult ovaries are relatively lighter than in the late fetus but the testes are heavier, or 9.16 times the absolute weight of the ovaries in the adult.

SUMMARY

Empirical formulae are given whereby the weight of any one of the four organs of the fetal urogenital system may be determined from either the body weight or the body length. The average weights in grams and the average relative weights are given for the adult organs, and also formulae are given for the more accurate calculation of the weights from the adult body weight.

The fetal kidneys form curves concave superiorly when plotted on either body weight or body length. Their percentage weight increases rapidly at first and then more slowly to its maximum in the late fetus. It forms a slightly smaller percentage of the body weight in the adult. The male kidneys are significantly heavier. There is no significant difference in the weight of right and left kidneys, but the right kidney is usually at a higher level in both fetal and adult cats. The kidney has good correlations with body weight and fair correlations with body length. It is fairly well correlated with the other parts of this system.

The bladder was not weighed in the fetal cats, but it is significantly heavier and more variable in the male cats. It forms 0.10 per cent of the body weight in both sexes. The male bladder is well correlated with body weight, body length and with the other organs of the urogenital system.

The testes form straight line curves on both body weight and body length with the exception of the initial part of the curve on body weight. They decrease from about 0.11 per cent of the body weight in the early fetus to 0.01 in the late fetus and rise to 0.07 per cent in the adult. The left testes seem to migrate a little earlier, but both glands enter the scrotum just about the time of birth. The adult testes are the most variable of these organs in weight. There seems to be no significant

difference in weight between the right and left testis. All of the correlations with the adult testes are good.

The ovaries form a straight line when plotted on body weight and a line concave superiorly when plotted on body length. In the early fetus they form 0.11 per cent of the body weight, decreasing to 0.016 per cent in the late fetus and to but 0.009 per cent in the adult. There is possibly a slight asymmetry in the fetal ovaries with the right heavier and in the adults the right ovary is heavier more frequently but its average weight is not greater. They have significant correlations with only the body weight and the kidneys.

The fetal growth curves of the uterus are both concave superiorly. It increases in percentage of the body weight during the fetal period and the adult percentage is about the same as in the late fetal period. The uterus has the highest coefficient of variation of any of the female organs in this report and it is second only to the testes in variability. It forms fairly good correlations with all of the parts except the ovaries and this correlation is positive but not significant.

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REGENERATIVE AND ACCESSORY GROWTHS IN PLANARIANS

I. Inhibition of the Development of Regenerative and Accessory Growths[1]

By

E. D. GOLDSMITH

Biological Laboratories, Harvard University, and Department of Biology, Washington Square College, New York University

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In a preliminary report (Goldsmith, 1934) it was stated that the data at present available from the field of experimental embryology and from the experimentation concerned with regeneration and regulation in the adult organism lead us to conclude that the animal which we call typical is not the maximum expression of all of its inherent formative potentialities. The facts necessitate the postulation of a mechanism whereby development is checked as well as one whereby it is initiated.

The experiments to be described in this paper were performed in an attempt to determine whether the phenomenon of the checking of development—inhibition—is an actuality in planarians.

The second paper in this series is concerned with the initiation of the development of supernumerary structures.

"INHIBITORY DOMINANCE"

Statement Of The Problem

1. To determine whether the phenomenon of "inhibitory dominance" really exists, i. e., would the conclusion derived from the variable observations made on a small number of the individuals hold if extended over a larger group?

2. To determine the "relative dimensional" factors involved. The term "relative dimensional" is here used to mean the quantitative and spatial relationships existing between the structure which is to be removed and those structures which remain, and the relation of the latter, especially a remaining head, to the cut surface.

The term regulation is here used to denote the return to a typical single-headed condition following the removal of one of the heads of a

1 Revision of a dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy at Harvard University.

double-headed individual. The term regeneration is used to denote the return of a head following head removal.

INTRODUCTION

Trembley (1744) split a hydra longitudinally and thus produced a double-headed hydra. In planarians double-headed individuals have been produced by Johnson (1835), van Duyne (1895), Randolph (1897), Morgan (1900, 1902), Bardeen (1901, 1903), and by numerous others during the past thirty-five years. The operation consists, essentially, of a longitudinal incision between the eyes. The regions may remain separated and each portion then regenerates the portion lacking, so that a double-headed animal ensues.

Utilizing this technique, Steinmann (1910) found that, following removal of one of the heads by cutting at a point close to the common body portion, a head did not regenerate, but a structure which he interpreted as tail-like resulted.

Rand and Ellis (1926), using specimens of Planaria maculata which had been made double-headed by a longitudinal splitting in the mid-line, performed a small number of operations in which one of the heads was removed by an incision made at the point of bifurcation or at some level distal to the common body. Of their results they state: "If, however, one of these duplicated parts be transected sufficiently close to the single common portion of the body, then (other conditions, not now to be specified in detail, being favorable) the regenerative replacement of that excised duplicate part may exhibit retardation or complete failure" (p. 574). In the same year, Rand and Browne (1926) in reporting a number of grafting experiments say: ". . . . regeneration of the planarian head may be inhibited by a foreign head brought, by grafting, into appropriate relation to the wound region of the beheaded worm" (p. 581).

From a series of grafting experiments on Hydra oligactis (fusca), Rand, Bovard, and Minnich (1926) concluded (tentatively) that the apical region of Hydra may act "to inhibit the operation of a developmental mechanism where such operation would result in a structure inconsistent with the attainment of a form normal in relation to the dominant head region" (pp. 569-570).

Shortly after the publication of the above results the work (preliminary report, Goldsmith, 1930, 1934) described in the following section was undertaken.

EXPERIMENTAL

In order to ascertain the existence of "inhibitory dominance" and the "relative dimensional" factors concerned in its manifestation, planarians with supernumerary heads were used as experimental material. The procedure consisted in varying the absolute and relative size of the heads of artificially produced double-headed individuals and in making the incision for removal of one of the duplicated structures at different levels so that varying lengths of the duplicated portions remained.

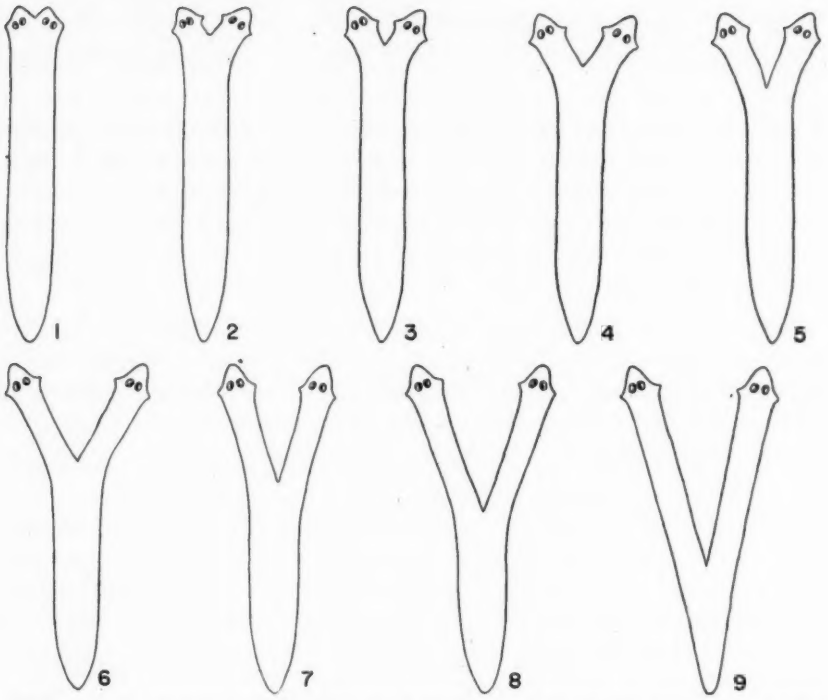
Since in all previous experimentation on double-headed planarians, so far as I am aware, little attention was paid to the relative size of the parts involved, it was desirable to obtain a series in which the relations of size and space were determined by careful measurements.

Euplanaria novangliae[2] (Planaria maculata), collected in the ponds in the vicinity of Waverley and Woods Hole, Mass., were used. The operations were performed under a binocular dissecting microscope. The animals were very lightly anesthetized in 0.1% chloretone, and then placed in a Syracuse watch glass which had been partially filled with paraffin. They were split longitudinally either in the mid-line or at one side of the mid-line, depending on whether heads of equal or unequal size were desired. The length of the incision was varied. Since each portion resulting from the splitting operation regenerated an approximate equivalent of its own bulk, individuals with varying relative sizes of duplicate parts were obtained (figures 1-9). These double-headed forms comprising the experimental material were kept in group cultures and were fed beef liver several times a week. Several days before the secondary operations the animals were placed in individual aquaria.

Prior to the secondary operation the worms were carefully measured as they moved on a cover slip placed over a piece of millimeter-ruled cross section paper. The same measuring device was used throughout the investigation. The observations were made with the aid of a

2. After this paper had gone to press, Dr. Libbie Hyman informed me in a private communication that the name Dugesia tigrina (Girard) 1850 is the correct one. In previous papers I have used the name Planaria maculata (cf. Hyman, 1931). Other synonyms which occur in the literature are Planaria lata, Euplanaria maculata and Euplanaria tigrina.

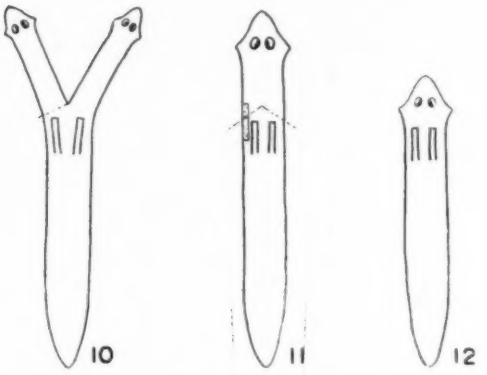
Procotyla fluviatilis has erroneously been called Dendrocoelum lacteum. D. lacteum is a European form.



Figures 1-9. Outlines of double-headed individuals of Euplanaria novangliae to show relative sizes of duplicated parts. All of the cases listed in Tables I and II fall within these limits. The ratios of the total lengths (T. L.) of the animals to the lengths of the duplicated parts (H1) follow:

Fig. 1. "Fused" type; Fig. 2. 10:1; Fig. 3. 8:1; Fig. 4. 5.1:1; Fig. 5. 4.1; Fig. 3:1; Fig. 7. 2.7:1; Fig. 8. 2.1:1; Fig. 9. 1.5:1.

binocular dissecting microscope. After being measured, the animal was placed in the paraffined operating dish and the desired length of the duplicated region was removed by an incision made with a sharp cataract knife (fig. 10). The animal was reexamined to verify the level of transection, and then isolated in a finger-bowl containing tap water. The planarians were thereafter examined periodically. The first appearance of eye pigment, eye vesicles, and the shape of the head were used as criteria for regeneration time. Whether the regenerated head was typical or aborted and whether the regeneration proceeded at a normal or retarded rate were noted. If, following removal of the head, regeneration of a head resulted, then in many cases the animals were again measured, and either the regenerated or the old head was



Figures 10-12. Outline drawing of double-headed planarian in which the left head is removed (fig. 10). The planarian has regulated to become typically single-headed. The remaining head is removed by a Λ-shaped cut (fig. 11) as explained, below. Symmetrical regeneration of a typical head follows (fig. 12).

In all figures broken lines indicate planes of cutting and stippling (except in figs 31 and 32) indicate a zone in which head regeneration has not followed head removal.

removed. In those cases in which, following removal of one head, regeneration did not occur, the regenerative powers of the region of failure were tested by making a V-shaped incision with the apex of the V directed towards the apical end of the worm (fig. 11) and so placed that one of the arms of the cut which removed the remaining head passed through the region which had failed to respond to the previous cutting. If a head now developed (fig. 12) it could be safely concluded that the former failure was not due to lack of regenerative potency.

About two hundred and twenty-five animals were measured and treated in the manner described.

In view of the results obtained as the investigation progressed it became evident that the following "relative dimensional" factors were important:

1. Body level (level on antero-posterior axis at which the incision is made removing one of the two heads).
2. Distance (length of the duplicated part plus the length of the stump).
   a. Length of stump (length of duplicated region left projecting beyond the common posterior part of body).

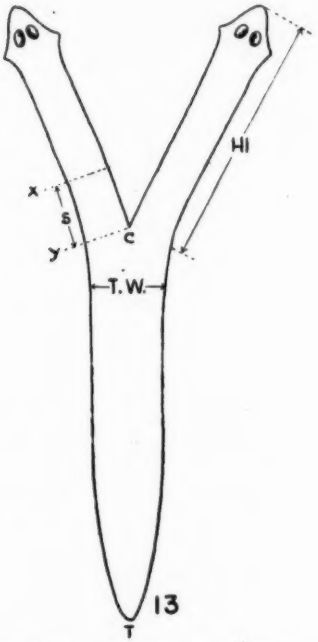


Fig. 13. Outline of a double-headed planarian illustrating transections at two levels, one through Y removing the entire duplicated part and another through X removing a portion of the duplicated region below, and (cf. text p. 125, line 22.

Symbols: C = point at which duplicated parts join the common posterior body; T = posterior tip of tail; others as in legend in Table I.

The secondary and tertiary operations, designed to discover the relative importance of these factors, involved head removal in animals with equal or unequal heads and at different levels of the antero-posterior axis. In one group the incisions were made at the level at which the duplicated anterior ends joined the common posterior body (fig. 13), so that the duplicated region was removed entirely; in these cases stump length is zero. In the second group the level of transection was distal to the common body region (fig. 13) so that a portion of the duplicated region was left; here stump length is greater than zero.

Case histories of a number of representative forms illustrating significant points are given. The cases have been arranged in tables so that the "relative dimensional" factors involved would be open to analysis.

TABLE 1

DUPLICATED PARTS EQUAL IN SIZE.* STUMP LENGTH A CONSTANT, 0

Case No.	Total Length (in mm.) T. L.	Width of Body (in mm.) T. W.	Length of Duplicated Part (in mm.) RHl	Width of Duplicated Part (in mm.) RHw	(Level of Cut) Ratio of Total Length to Length of Duplicated Part T.L. :Hl	Distance (Stump + Length of Duplicated Part) S + Hl	Duplicated Part Removed	Result
A3	8.0	0.7	0.8	0.5	fused**	—	LH	2e.
A3a	7.0	0.6	0.7	0.7	(Is but a single head with 4 eyes and half of head)		Removed 2 eyes.	N.R.
B1	7.0	0.9	1.0	0.6	fused	—	LH	N.R.
P2	7.0	0.8	0.8	0.6	fused	—	RH	R.5.
U3	7.0	—	0.5	0.6	fused	—	LH	N.R.
1–15	6.7	—	0.7	0.4	fused	—	RH	N.R.
U8	6.5	—	0.3	0.7	fused	—	LH	N.R.
2–5	6.0	—	0.8	0.7	fused	—	LH	1e.15;2e. a.24.
B4	6.0	—	0.8	0.4	fused	—	RH	N.R.
P4	6.0	0.8	0.4	0.5	fused	—	RH	1e. 9.
P6	6.0	0.8	0.6	0.6	fused	—	LH	N.R.
D17	6.0	0.5	0.4	0.4	fused	—	RH	N.R.
U10	6.0	—	0.3	0.4	fused	—	RH	N.R.
D10	5.7	0.5	0.4	0.4	fused	—	LH	N.R.
215	5.0	1.3	0.8	1.0	fused	—	LH	N.R.
4–16	5.0	1.0	0.4	0.6	fused	—	RH	1e. 12.
3–17	5.0	0.7	0.7	0.5	fused	—	RH	N.R.
P8	5.0	0.4	0.7	0.4	fused	—	LH	1e.
E3	5.0	(no other measurements)			fused	—	RH	N.R.
D18	5.0	0.5	0.4	0.3	fused	—	RH	1e.
1–13a	5.0	(no other measurements)			fused	—	RH	1e.
E4	4.8	0.8	0.3	0.3	fused	—	LH	N.R.
P5	4.5	0.5	0.6	0.4	fused	—	RH	N.R.
3–18	3.5	0.6	0.5	0.5	fused	—	RH	er.
U22	3.5	—	0.3	0.4	fused	—	LH	N.R.
E6	3.0	0 5	0.3	0.3	fused	—	RH	N.R.
D21	3.0	0.4	0.4	0.3	fused	—	RH	N.R.
E5	5.5	0.6	0.5	0.4	11:1	0.5	RH	N.R.
G1	3.3	0.5	0.3	0.3	11:1	0.3	RH	N.R.
4–4	9.0	1.0	0.9	1.0	10:1	0.9	LH	2e.a.12.
200	8.0	1.1	0.8	1.0	10:1	0.8	LH	R.4.
200a	(Animal smaller than 200)			—	fused	—	LH	N.R.
U20	3.0	—	0.3	—	10:1	0.3	RH	N.R.
1–13	7.5	1.0	0.8	0.8	9.3:1	0.8	LH	R.15.
U2	6.0	—	0.7	—	8.5:1	0.7	RH	N.R.
D5	6.3	—	0.8	—	7.8:1	0.8	LH	N.R.
EE4	4.6	0.8	0.6	0.7	7.6:1	0.6	RH	N.R.
4–5	7.5	1.2	1.0	0.9	7.5:1	1.0	LH	N.R.
5–7	8.1	1.1	1.1	0.9	7.4:1	1.1	RH	R.10.
1–16	8.5	1.0	1.2	1.0	7.1:1	1.2	LH	R.13.
2–6	9.1	1.3	1.3	1.1	7:1	1.3	RH	R.15
1–12	7.0	1.1	1.0	0.8	7:1	1.0	RH	1e.9.
U21	4.0	—	0.5	—	6.6:1	0.5	LH	N.R.
J2	6.5	—	1.0	—	6.5:1	1.0	LH	N.R.
4–18	6.5	1.1	1.0	0.9	6.5:1	1.0	LH	R.8–9.
4–9	8.3	1.0	1.3	1.0	6.4:1	1.3	RH	R.12.
1–17	8.0	1.1	1.3	1.0	6.1:1	1.3	RH	R.7.
1–10	6.0	1.2	1.0	1.0	6:1	1.0	LH	2e.
1–11	6.0	1.3	1.0	0.9	6:1	1.0	RH	R.9.
4–10	6.7	1.0	1.2	0.7	5.6:1	1.2	LH	R.12.
1–26	5.0	—	1.0	0.8	5:1	1.0	RH	2er.14.
1–18	9.5	1.2	2.0	0.9	4.7:1	2.0	RH	R.10.
D6	4.2	0.6	0.9	0.6	4.6:1	0.9	LH	N.R.
4–15	8.5	1.0	1.9	1.0	4.5:1	1.9	RH	R.6.
E2	5.8	0.5	1.3	0.3	4.5:1	1.3	RH	N.R.
2–4	6.5	0.8	1.5	0.8	4.3:1	1.5	LH	er.15.
2–2	8.2	1.0	2.0	1.0	4.1:1	2.0	RH	R.8.
4–8	8.0	1.0	2.0	0.9	4·1	2.0	RH	2e?r24.
EE2a	6.0	1.4	1.5	0.6	4:1	1.5	RH	N.R.
U9	5.3	—	1.3	—	4:1	1.3	RH	N.R.
1–22	8.9	1.0	2.4	0.8	3.7:1	2.4	RH	1e?12.;2ea.17.
1–7	6.9	0.9	1.9	0.7	3.6:1	1.9	LH	N.R.
3–13	5.6	1.0	1.6	0.5	3.5:1	1.6	RH	N.R.
5–2	7.0	0.8	2.0	1.0	3.5:1	2.0	LH	R.12.
1–27	8.4	—	4.2	0.8	3.4:1	2.4	RH	N.R.
3–8	6.8	0.9	2.0	0.6	3.4:1	2.0	RH	N.R.
J1	4.5	—	1.3	—	3.4:1	1.3	RH	N.R.
3–5	7.2	0.9	2.2	0.7	3.3:1	2.2	LH	R.4.
K1	8.0	1.1	2.5	1.0	3.2:1	2.5	LH	N.R.
3–12	5.5	0.6	1.7	0.4	3.2:1	1.7	RH	N.R.

* See Figures 1-9 for types of double-headed planarians.

** See Fig. 28 for the type of cut made for head removal in "fused" heads.

TABLE 1 (Cont'd)

Case No.	Total Length (in mm.) T. L.	Width of Body (in mm.) T. W.	Length of Duplicated Part (in mm.) RH1	Width of Duplicated Part (in mm.) RHw	(Level of Cut) Ratio of Total Length to Length of Duplicated Part T.L. :H1	Distance (Stump + Length of Duplicated Part) S + H1	Duplicated Part Removed	Result
3–7	6.0	0.7	2.0	0.6	3:1	2.0	RH	1e.14.; 2ea.24.
1–24	6.0	1.0	2.2	0.9	2.7:1	2.2	RH	1e?13; 1ea.18.
1–19	6.0	1.0	2.3	0.6	2.6:1	2.3	LH	N.R.25.
1–23	5.7	0.8	2.2	0.6	2.6:1	2.2	RH	N.R.30.
3–9	5.3	0.7	2.0	0.6	2.6:1	2.0	RH	R.12.
EE2	5.3	0.6	2.3	0.5	2.3:1	2.3	RH	N.R.
EE1	5.5	1.1	2.5	0.5	2.2:1	2.5	LH	N.R.
3–1	6.0	0.6	3.0	0.5	2:1	3.0	RH	N.R.
1–5	6.0	0.8	2.9	0.8	2:1	2.9	RH	N.R.
4–1	4.0	—	2.0	—	2:1	2.0	RH	N.R.
U1	4.0	—	2.0	—	2:1	2.0	RH	N.R.
3–4	6.1	0.8	3.3	0.6	1.9:1	3.3	RH	N.R.
5–1	7.0	1.0	4.0	0.6	1.7:1	4.0	LH	N.R.
N1	8.0	1.2	5.0	1.0	1.6:1	5.0	LH	N.R.
1–4	6.0	1.0	4.0	—	1.5:1	4.0	RH	N.R.

The cases are arranged on the basis of level of transection beginning with the most anterior (fused) and ending with the most posterior level (1.5:1).

Symbols: T. L. = total length of planarian as measured from the tip of the head to the tip of the tail; T. W. = width of body as measured one mm. posterior to the point of union of the two duplicated regions; H1 = length of the duplicated part, i. e., one of the heads plus the duplicated region posterior to it; Hw = width of the duplicated part at the level of and including the auricles; RH = right duplicated part; LH = left duplicated part; S = length of the stump remaining after removal of a portion of one of the duplicated parts; T. L.:H1 — S = the level of the cut computed by dividing the total length of the planarian by the length of the duplicated part minus the length of stump; T. L.:H1 = as the preceding, here the stump length is zero; fr. = stump less than 0.1 mm. in length; R = regeneration of a typical head; a = regeneration of an aborted head; N. R. = no regeneration of a head either typical or aborted; animal regulates to become a typical single-headed individual. 1e. = development of an additional eye usually in region posterior to one of the original eyes of the remaining head; 2e. = development of two additional eyes in the remaining head; er. = development of a single eye in a small amount of regenerated tissue; 2 er. = development of two eyes in a small amount of regenerated tissue; e? = possible eye, the vesicle (clear area) is not visible.

A semi-colon separates the records of an earlier and later observation. A number after any of the above in the "Result" column indicates time in days.

ANALYSIS OF DATA IN TABLE I

(DUPLICATED PARTS EQUAL IN SIZE. STUMP LENGTH A CONSTANT, 0)

(Total, 85 cases)

Level of Transection	Result	Number of cases
Heads fused 28 cases	Regeneration of a typical head	1
	Development of one additional eye in regenerated tissue	4
	Development of two additional eyes in old tissue	1
	Development of one additional eye in old tissue	4
	Regulation	18
11:1—7:1 14 cases	Regeneration of a typical head (three at a retarded rate)	5
	Regeneration of an aborted head (at a retarded rate)	1
	Development of one additional eye in old tissue	1
	Regulation	7

Level of Transection	Result	Number of cases
6:1—5:1 9 cases	Regeneration of a typical head (one at a retarded rate)	5
	Development of two eyes in a small amount of tissue (at a retarded rate)	1
	Development of two additional eyes in the remaining head which became double	1
	Regulation	2
4:1 9 cases	Regeneration of a typical head	3
	Development of two eyes in regenerated tissue (at a retarded rate)	1
	Development of one eye in a small amount of regenerated tissue (at a retarded rate)	1
	Regulation	4
3:1—2.6:1 15 cases	Regeneration of a typical head (one small and at a retarded rate)	4
	Development of two eyes in a small amount of regenerated tissue (at a retarded rate)	1
	Development of a single eye (at a retarded rate) in old tissue	1
	Regulation	9
2.3:1—1.5:1 10 cases	Regulation	10

TABLE II

DUPLICATED PARTS EQUAL IN SIZE. STUMP LENGTH A VARIABLE.

Case No.	Total Length (in mm.) T.L.	Width of Body (in mm.) T.W.	Length of Duplicated Part (in mm.) Hl	Width of Duplicated Part (in mm.) Hw	Stump Remaining S	(Level of Cut) Ratio of Total Length to Length of Duplicated Part Minus Stump Length T.L.: Hl-S	Distance (Stump + Length of Duplicated Part) S + Hl	Duplicated Part Removed	Result
D7	6.2	—	0.7	0.5	0.2	12.4:1	0.9	RH	R.18.
J5	6.6	0.7	0.6	0.4	fr.	11:1	0.6	LH	R.15-20.
D15	7.0	0.7	0.7	0.7	fr.	10:1	0.7	LH	R.10.
D11	4.5	0.5	1.0	0.5	0.5	9:1	1.5	RH	le.14.
4–11	7.9	1.3	0.9	1.0	fr.	8.8:1	0.9	LH	le.
201	6.0	1.1	1.1	1.0	0.4	8.6:1	1.5	LH	R.5.
H11	5.5	0.7	0.7	0.4	fr.	8:1	0.7	RH	N.R.
U15	7.0	1.0	1.0	0.6	0.1	7.8:1	1.1	RH	N.R.
202	6.0	0.9	0.8	0.8	fr.	7.5:1	0.8	LH	R.a.7.
D12	4.4	0.5	1.4	0.4	0.7	6.3:1	2.1	LH	le.8.
201a	6.0	—	1.1	—	fr.	5.4:1	1.1	LH	R.5.
4–13	6.6	1.0	1.6	0.9	0.1	4.4:1	1.7	LH	le.12.;R,15.
5–6	10.0	1.2	3.0	0.9	fr.	3.3:1	3.0	RH	R.10
4–3	6.5	1.0	2.0	1.0	fr.	3.2:1	2.0	RH	R.10.
4–7	6.1	1.0	2.6	0.8	0.5	2.9:1	3.1	RH	R.14.
D3	5.0	—	2.0	0.5	0.2	2.8:1	2.2	LH	R.13.
5–5	7.0	0.9	3.0	0.5	0.4	2.7:1	*3.4	LH	R.10.
D1	5.0	0.8	3.0	0.5	0.8	2.3:1	3.8	RH	N.R.
1–20	7.5	0.7	4.0	0.6	0.3	2:1	4.3	LH	R.16-18.
3–2	7.0	0.6	4.0	0.7	0.2	1.8:1	4.2	RH	N.R.
P1	6.5	0.7	5.0	0.7	1.0	1.6:1	6.0	LH	R.7.

ABBREVIATIONS AS IN TABLE I.

CASE HISTORIES

Non regeneration in the presence of a pharynx

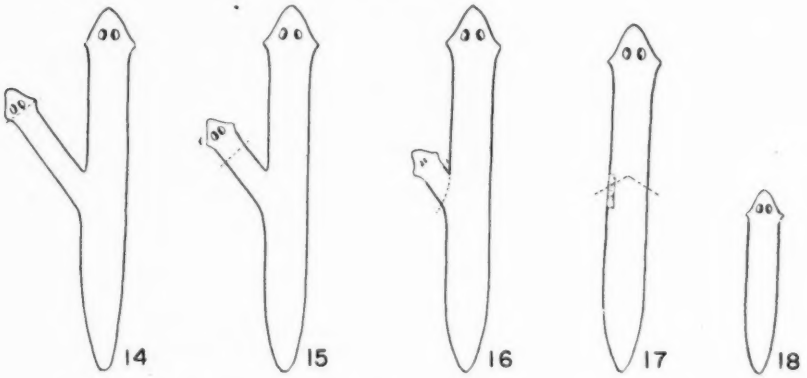
Case 1-7

March 15. Animal measures:
T. L. 6.9 mm.; RH1 1.9 mm.; LH1 1.9 mm.
T.W. 0.9 mm.; RHw 0.7 mm.; LHw 0.7 mm.
Ratio T. L.:H1 is 3.6:1.
Removed left hand by oblique cut with no stump remaining (fig 10). A pharynx lies directly posterior to the cut surface; a second pharynx is present (fig. 10).

March 24. Animal is typically single-headed. Two pharynges are present.

April 10. Removed the single head by a V-shaped incision involving that portion which had formerly failed to regenerate (fig. 11).

April 18. T. L. 3 mm. A well developed head with typical eyes has regenerated fig. 12).



Figures 14-18. The importance of the stump in head regeneration following head removal in double-headed planarians, as explained below.

Importance of the Stump

Case 207

July 7. Animal measures:
T. L. 7.0 mm.; RH1 3.2 mm.; LH1 1.9 mm.
T. W. 1.2 mm.; RHw 1.2 mm.; LHw 0.7 mm.
Ratio T. L.:LH1 — S is 10:1.
Left head is smaller head but is very active. Removed left head by an oblique cut directly posterior to the eyes (fig. 14).

July 11. Eyes present in the regenerated tissue of the stump of left head.

July 21. Removed left head by an oblique cut leaving a smaller stump (fig. 15) than previously (approximately 0.4 mm. in length).

July 24. Minute eyes present in the regenerated tissue of stump of left head. Removed regenerated left head leaving no stump (fig. 16).

July 29. Animal typically single-headed. Removed remaining head, at level where regeneration has not resulted previously, by a V-shaped incision with the apex of the V directed towards the anterior end of the worm (fig. 17).

Aug. 1. Symmetrical regeneration. Eyes present in the new head (fig. 18). Animal measures:
T. L. 3.5 mm.; T. W. 0.6 mm.

Retardation and aborted development

Case D 9

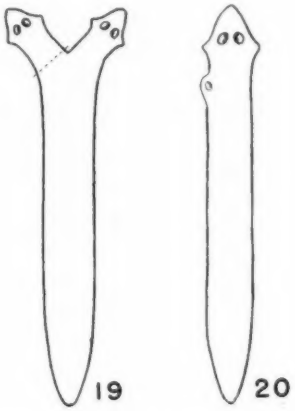
Jan. 19. Animal measures:

T. L. 4.6 mm.; RH1 0.6 mm.; LH1 0.6 mm.

T. W. 0.6 mm.

Ratio T. L.:H1 — S is 9:1.

Removed left head by an oblique cut leaving a stump slightly less than 0.1 mm. (fig. 19).



Figures 19-20. Aborted development following removal of one of the heads in a doubled-headed planarian as explained above (Case D9).

Feb. 6. A pigment spot first observed on Feb. 2, fourteen days after the decapitation, now has a curvature similar to that of the pigment cup in a typical left eye and lies in a vesicle (fig. 20).

Note: A second eye never developed.

Importance of the head in non-regeneration

Case P 1

Oct. 29. Animal measures:

T. L. 6.5 mm.; RH1 5.0 mm.; LH1 5.0 mm.

T. W. 0.7 mm.; RHw 0.7 mm.; LHw 0.7 mm.

Ratio T. L.:LH1 — S is 1.6:1.

Removed left head by an oblique cut leaving a stump of 1.0 mm. (fig. 21).

Nov. 4. Incipient eyes present in regenerated tissue at the distal end of the stump.

Nov. 6. Eyes now typical in regenerated head (fig. 22).

Animal measures:

T. L. 6.0 mm.; RH1 4.8 mm.; LHL 1.3 mm.

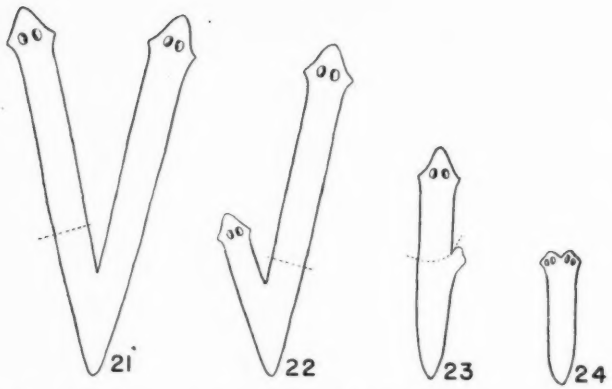
T. W. 0.6 mm.; RHw 0.6 mm.; LHw 0.3 mm.

Ratio T.L.:RH1 — S is 1.4:1.

Removed right head leaving stump 0.6 mm. in length (fig. 22).

Remaining left head controls direction of movement; stump attempts to move in a contrary direction.

Nov. 8. Slight regeneration of tissue at distal end of stump.

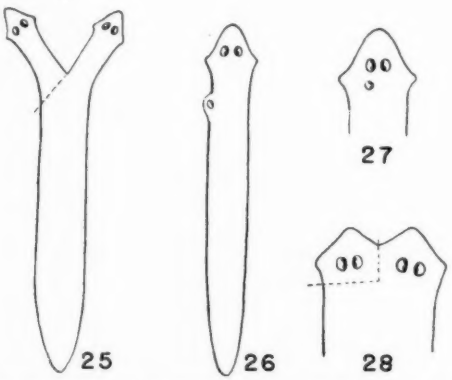


Figures 21-24. Importance of the remaining head in non-regeneration following removal of one of the heads in a double-headed planarian as explained, p. 119, line 14 of text (Case P1).

Nov. 14. No additional regeneration since Nov. 8. Pricked distal end of stump with cataract knife.

Nov. 18. No trace of injury; repeated injury operation.

Nov. 21. No sign of regenerated tissue at anterior end of stump.
Animal measures:
T. L. 4.0 mm.; RH1 (stump) 0. 25 mm.; LH1 2.0 mm.
T. W. 0.5 mm.; RHw (stump) 0.3 mm.; LHw 0.4 mm.
Removed left head leaving no stump (fig. 23). Injured tip of stump of right head as previously.



Figures 25-27. Eye regeneration, resorption of tissue and change in position of eye as explained above.

Fig. 28. Outline drawing of "fused" heads showing where cuts for removal of one of the heads are made.

Nov. 25. Regenerated tissue present at both surfaces.

Nov. 27. Two minute pigment spots resembling eye pigment are present in stump of right head. Left head is regenerating.

Nov. 29. Two eyes observed in right head; one eye in left head.

Dec. 23. Two eyes present in left head (fig. 24).

Resorption

Case 2-4

March 28. Animal measures:

T. L. 6.5 mm.; RH1 1.5 mm.; LH1 1.5 mm.

T. W. 0.8 mm.; RHw 0.6 mm.; RHw 0.6 mm.

Ratio T. L.:H1 is 4.3:1.

Removed left head by oblique cut leaving no stump (fig. 25).

April 16. A single eye visible, in a mass of regenerating tissue projecting laterally (fig. 26).

June 3. Projecting mass of regenerating tissue is no longer present. The animal is typical in outline but a supernumerary eye (left eye from the curvature of the pigment mass) lies posterior to the typical left eye (fig. 27).

Note: Resorption has been observed in planarians in which a stump remains following removal of one of the duplicated parts. In these cases regeneration does not take place; the stump is gradually resorbed and the animal becomes typically single-headed.

DISCUSSION

A study of Tables I and II, and of the individual case histories shows that following removal of one of the heads of a duplex individual regeneration may proceed at a greatly retarded rate and the result may be an aborted head. In numerous instances a head fails to regenerate and the animal regulates to become typically single headed. The question arises, are these cases of non-regeneration sporadic and accidental or may they be correlated with

1. The lack of regenerative potency?
2. The absence of those tissues which have been postulated to control or at least to exert a formative influence upon head regeneration in planarians, namely—
   a. The digestive system?
   b. The nervous system?
3. An "inhibitory dominance" which may be correlated with the remaining one of the former two head regions of the worm?

If this third possibility be the correct one can we further plot the factors which limit the operation of this inhibitory dominance?

Regenerative Potency

Planaria maculata typically exhibits a marked power of replacing lost parts. Morgan (1898) found that pieces 1/65, 1/100 and 1/279 of the weight of the original worm would regulate into a small somewhat typical individual. Li (1929) states that pieces constituting 1/30 of

the original individual of Planaria lugubris will form complete individuals. Curtis and Schulze (1934, p. 483) similarly report that individuals of Planaria maculata and Planaria agilis may be cut into twenty-five pieces with each piece forming a new individual.

Li (1929) has further demonstrated that successive decapitations, within limits, do not bring about loss of capacity for regeneration. He found that a typical head regenerated following nine decapitations performed at approximately ten-day intervals.

In none of the present experiments have the animals or the portions of animals even approached the small size of the fragments above described as being capable of regeneration. In addition, no animal was subjected to nine decapitations. However, most significant of all is the fact that regeneration followed the removal of the remaining head, as shown by the following experiment. After the excision of one head of a duplex individual and its subsequent failure to regenerate, the regenerative capacity was tested by removing the remaining head by a V-shaped incision with the apex of the V directed towards the anterior end (figs. 10 and 11) of the worm. This cut involved the region which had formerly failed to respond. Regeneration occurred, the amount of tissue regenerated was *equal* on the *two sides* and a symmetrical individual resulted from every such operation (fig. 12).

It is, therefore, evident that the explanation of "non-regeneration" does not lie in the lack of regenerative potentialities.

The Digestive System

The theory was advanced by Bardeen (1901) that the differentiation of "embryonic" (regenerating) tissue in Planaria maculata following injury depends upon the relation of this tissue to the "axial gut". Bardeen expressed the belief that the intestinal system has a specific formative influence on the "embryonic" tissue.

Lillie (1901) opposed this view, for he observed that in Dendrocoelum lacteum there was a region anterior to the pharynx in which "embryonic" tissue and a portion of the original axial gut were present two days after the operation and yet regeneration of a head did not take place. Beyer and Child (1930) believe that the alimentary tract is not of importance in the localization of the anterior end in Planaria maculata and Planaria dorotocephala.

The evidence here presented for Planaria maculata is of somewhat the same nature. Whether or not a head will regenerate following

removal of a head in a double-headed worm does not appear to depend upon the relation which the digestive tract bears to the cut surface. Cases have been noticed in which a pharynx was present in the region directly posterior to the cut surface (Case 1-7, p. 118). Others have been observed in which the pharynx was removed with the head so that the gut, formerly connected to the pharynx, was now in contact with the cut surface. In a third type only one pharynx was present, it being located in the midline. There appeared to be no correlation between the type or position of the pharynx and the regeneration of a head. Cases which were closely similar except for the position of the pharynx behaved in a similar manner.

It would seem that a pharynx in contact with a cut surface resulting from head removal might exert a mechanical stimulus. However, it did not exercise any observable formative influence.

The Nervous System

Whether the nervous system exerts a formative influence on head regeneration in planarians is a controversial matter. As previously shown Planaria maculata and Planaria agilis exhibit marked powers of head regeneration; whereas Dendrocoelum does not regenerate a head when more than the anterior third of the body is removed. Lillie (1901) attributed this difference in behaviour to increased cephalization in Dendrocoelum, and concluded that the presence of the brain and anterior portion of the central nervous system is the determining factor in head regeneration. Head regeneration, however, followed the removal of the whole of the cerebral ganglion and the lateral nerve cords in Dendrocoelum (Goldfarb, 1909). The absolute removal of all nerve cells was said to be impossible because of the diffuse character of the nervous system.

In marine polyclads (Child, 1904; Olmsted, 1922) and in a marine triclad, Gunda (Lloyd, 1914) anterior regeneration is dependent upon the presence of the cephalic ganglia.

Bardeen (1903) believes that the new ganglia are dependent upon the old nervous tissue. Flexner (1898), Keiller(1910) and Olmsted (1918) showed that in several fresh water triclads the cephalic ganglia arose in the new tissue independently of the old nervous system. Keiller observed that eyes not connected with the cephalic ganglia could develop. Beyer and Child (1930) regard the cephalic ganglia as

arising "independently of the parts of the old nervous system present in the piece". They conclude that the central nervous system may be a localizing factor but is not a determining factor in the formation of the head.

The experimental work reported within this paper indicates that the presence of a lateral nerve cord does not imply that head regeneration will follow head removal. In all cases following removal of a head, the lateral nerve cord was left in contact with the cut surface. Notwithstanding the presence of the nerve cord regeneration failed to take place in many cases.

Inhibitory Dominance

If the digestive and nervous systems do exert a "formative influence" then this influence is not sufficient to overcome some restraining influence or influences exerted by some other portion or constituent of the planarian body.

Child and Watanabe (1935) and Watanabe (1935) report that an inhibitory factor may originate at a posterior cut surface and inhibit regeneration at the anterior cut surface of short pieces of Euplanaria dorotocephala. They believe that this factor is transmitted, in part, by the nerve cords.

Since no tails were removed in any of the cases presented here the inhibitory factor must have arisen elsewhere than at a posterior cut surface. It appears that in these cases the inhibitory factor is somehow associated with the presence of the remaining head. The work presented in section, "Regenerative Potency" (p. 122) and cases like P_1 (p. 119, line 14) in which head regeneration does not proceed in the presence of a duplicated head but does follow when this duplicated head is removed, support this view.

The effectiveness of the head as an inhibitor is related to the "relative-dimensional" factors previously mentioned (p. 111, line 2; p. 113, line 13. These are now examined at greater length.

Body Level

In Table I are listed eighty-five cases. The right and left duplicated region of each individual were equal in size, but the individuals as wholes differed in size, and their duplicated parts differed accordingly in absolute size. Further, in consequence of the varying extent of the splitting of the animals, the size of the duplicated parts varied in relation to the size of the whole individuals. Since in all

cases ,the removal of one duplicate part was done so that no portion of the duplicated region remained, the level of transection may be expressed as the ratio between the total length of the animal and the length of the duplicated region. This relation is noted in the column termed "Ratio T. L.:H1". The higher the T. L.:H1 ratio the more anterior the level at which the cut, removing the duplicated part, was made. The series of figures (1-9) gives the range of duplicity in the double-headed forms.

From the analysis of the data (pp. 116, 117) presented in Table I it is evident that the regeneration of a head followed more frequently in the region lying between the levels 11:1 and 4:1 with the zone of highest frequency lying approximately between the levels 6:1 and 4:1. The level anterior to 11:1 and posterior to 4:1 manifested a lower frequency of head regeneration[3].

DISTANCE

The term "distance" as used in this paper is the distance in mm. from the anterior tip of the remaining head to the cut surface, measured as having two components (fig. 13); one, the length of the duplicated part and the other, the length of the stump. If the left duplicated part were removed at the level x indicated in figure 13, then the "distance" would be equal to the length of the right duplicated part (distance in mm. from the tip of the right head to the point of union, c, of the right and left duplicated regions) plus the length of stump (distance from c to the level of transection, x).

In the series where the stump length is zero (Table I) we are dealing with but one of the components of "distance", namely the length of the duplicated part. This component may vary widely from case to case and yet the end result may be the same. At levels 2.3:1 to 1.5:1 (Cases EE2, etc. Table I), the distance from the remaining head to the cut surface varies from 2.3 to 5.0 mm.; nevertheless, all cases behaved similarly in that regeneration did not occur. Of the eighty-five cases listed in this table fifty regulated to become single-headed animals following removal of a head.

In Table II (p. 117) are those cases in which the stump length was a variable, something greater than zero. Complete regulation followed re-

3. There is a striking agreement between these results and the relative distribution of formative cells as demonstrated by Curtis and Schulze (1934, fig 11). The relationship of the two sets of data and its possible significance will be discussed in a forthcoming paper.

moval of a head in only four of the twenty-one cases listed. Thus, although the number of cases is small, it is evident that the frequency of regeneration in these animals is higher than in those listed in Table I.

It is realized that by increasing the length of the stump one moves to a more anterior level at which there is higher regeneration frequency. The level of transection in these cases, where a portion of the duplicated region remained, is therefore expressed as the ratio between the total length of the animal and the length of the duplicated region minus the length of the stump. This relation is noted in the column (in Table II) termed "Ratio T. L.:H1-S" and is comparable to the "Ratio T. L.:H1" as used in Table I where the stump length is zero. A comparison of the data in Table I and the "Analysis of the Data of Table I" with the data in Table II shows that for similar zones the frequency of head regeneration is greater when a stump is left than when the cut is made so that no stump remains. Further, when a stump was left, heads have regenerated at posterior levels; Cases 1-20 and P_1 (Table II), where the "T. L.:H1-S Ratio" are 2:1 and 1.6:1 respectively, are cases in point. In no case in which the stump length was zero (Table I) did a head regenerate when the level at which the cut was made ranged form 1.5:1 to 2.3:1.

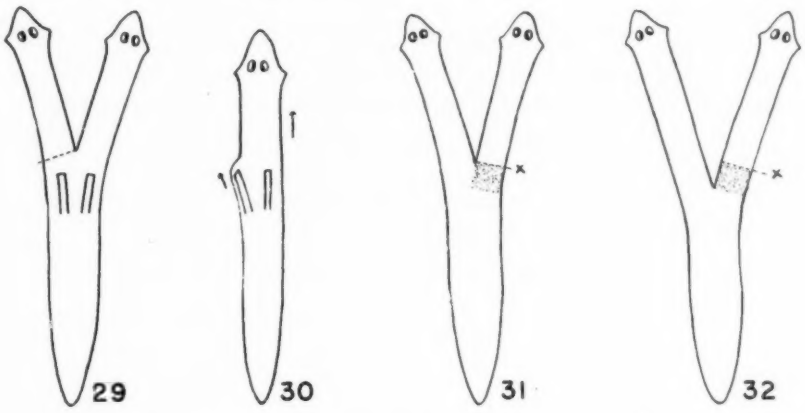
Since the other factor of distance, distance in mm. from the tip of the remaining head to the cut surface, has been ruled out (p. 125, lines 29-37) it would appear that the "stump" component is the important one. Further evidence in support of the view that the stump in itself is important is offered by cases like 207 (p. 118) in which regeneration follows removal of a head when a stump is left, but does not follow when a stump is not left.

The reason for the stump's importance can at present only be surmised. The following may be advanced as possible explanations:

1. The stump may make the region less accessible to the inhibiting agent. This does not appear to be very likely.

2. When a duplicated part is removed so that a stump remains the anterior end of the stump may become the "dominant" region of a new gradient (Child, Physiological Gradients). When the cut is made so that no stump is left (stump length is zero) the cut surface may become a "dominated" portion of the antero-posterior gradient extending from the remaining head to what had previously been the common tail. However, I have observed that the region posterior to the level of tran-

section may continue to contract as if it were attempting to move in a direction contrary to that of the remaining head (figures 29 and 30). Prior to section the duplicated parts had at times moved in opposite directions. It is thus possible that the cut surface becomes the new



Figures 29-30. Continued activity of region posterior to the level of transection as explained above. Arrows indicate directions of movement or attempted movement.

Figures 31-32. The cuts indicated by x and x are at the same level on the antero-posterior axis. The region (stippled) directly posterior to the level of transection in fig. 31 lies in the common posterior body, whereas, in fig. 32 the region (stippled) directly posterior to the level of transection extends anteriorly beyond the common posterior body (text, p. 128, line 1).

anterior end of the gradient which had formerly extended from the duplicated head (now removed) to the tail as shown in figure 30. Regardless of what the "physiological gradient" interpretation may be, the data do indicate that the results obtained when stumps are left differ from those obtained when no stumps are left. If this difference is to be attributed to a difference in the "physiological gradient" the mechanism of the inhibition still evades us.

3. In a forthcoming paper attention is called to "formative cells" (Curtis et al.), and their place in the regenerative scheme is discussed. The number of "formative cells" may be higher in the stump region than it is in a corresponding region which is an integral part of the common body (figures 31 and 32), although both regions are at the same level on the antero-posterior axis.

In conclusion, it may be said that the phenomenon of "inhibition"

demonstrated in planarians is not without precedent in other organisms. It is of interest to note that Murphy (1937) has reported that normal mammalian tissues (placenta and embryonic skin) contain a factor which definitely inhibits the growth of both transplanted and spontaneous cancers of animals.

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SUMMARY

1. The data from a large number of cases clearly demonstrated that the "inhibitory dominance" postulated by Rand and his coworkers from the results obtained from a small number of planarians holds. This "inhibitory dominance" may manifest itself not only in the non-regeneration which may follow removal of one head in double-headed planarians but also in the regeneration of a head at a retarded rate and in the regenertion of an aborted head.

2. These failures of typical head regeneration are not to be attributed to a lack of regenerative potency nor to the absence of tissues (such as those composing the digestive or nervous systems) which have been said to control or exert a formative influence upon head regeneration in planarians.

3. It has now been shown, both directly and indirectly, that the non-regeneration or the atypical regeneration which may follow removal of one head in double-headed planarians is to be attributed to the presence of the remaining head.

4. The effectiveness of the remaining head as an inhibitor is related to certain "relative dimensional" factors (p. 109, line 20) such as,—the body level at which the transection is performed, and the stump component of the distance factor. The other components of distance—length of the duplicated part (H1, fig. 13)—is relatively unimportant at a posterior level. It is difficult to isolate the various factors at anterior levels.

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BIOLOGICAL DIFFERENCES IN INDUCED MOUSE SARCOMATA, DISCLOSED BY THEIR GROWTH ENERGY IN ALIEN STRAIN MICE[1]

By
MARGARET R. LEWIS
and
EDNA G. LICHTENSTEIN
Department of Embryology, Carnegie Institution of Washington
(Received May 1, 1939)

It has been pointed out in previous publications (M. R. Lewis, 1937, 1, 2; Lewis and Lichtenstein, 1936) that the transplantable sarcomata, brought about in mice of pure inbred strains by the injection of 0.8 mg. 1:2:5:6—dibenzanthracene dissolved in oil, differ biologically even when histologically alike. One of these differences lies in the amount of myeloid hyperplasia that develops in the host during the growth of the tumor; another, in the ability of certain of the tumors to overcome to some extent the resistance of mice of pure inbred strains to the growth of an implant of a tumor that originated in a mouse of another strain. A third difference is shown by the inherent power of grafts of certain tumors to grow to some extent in mice of any strain, followed by a regression of the growth except in mice of the strain in which the tumor originated.

In an earlier communication (Lewis and Lichtenstein, 1937) the transplantability of 200 dibenzanthracene tumors induced in mice of pure inbred strains was described. Since that time 94 additional tumors have been induced and their transplantability in mice of the inbred A, BA, C3H and C57 strains has been studied. All of them proved to be 100 per cent transplantable into mice of the strain in which the tumor originated. Five were found to be tumors whose strain specificity could be overcome, so that they became transplantable into mice of alien strains. Three were regressing tumors; that is, when transplanted into mice of other strains they grew and then regressed. Therefore, among the total 294 induced dibenzanthracene tumors (Nos. 1 to 294) we identified 19 that, by experimental procedure, could be rendered transplantable into mice of many strains, and 7 that were regressing tumors; that is, while explants of these tumors grew in many strains of mice, the tumors thus formed regressed in all the hosts except those of the strain in which the tumor originated.

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The 19 non-strain specific tumors and the 7 regressing tumors were of the myeloid stimulating type; which is to say, a severe myeloid hyperplasia developed in the host coincident with the growth of a graft of one of these tumors.

At the time this investigation was undertaken 6 of the regressing tumors (BA-1, BA-178, BA-193, C57-220, C57-241 and C3H-263) were available for study. These were spindle-cell sarcomata, which, in stained preparations, appeared to be alike. The letters designate the strains of mice in which the tumors originated and into which they were thereafter serially transplanted; the numbers show the order in which the tumors arose. A tumor transplanted serially into mice of the same strain in which the tumor was induced is called a *straightline* tumor. Small fragments of each primary tumor that appeared at the site of the dibenzanthracene injection were grafted subcutaneously into the right and left axilla of two normal young mice of the same strain as the one in which the tumor developed. When these implants had grown into moderately large tumors (20x20x10 mm.) one host was sacrificed and fragments of its tumor were transplanted into two normal young mice of the same strain, the other host being kept until it succumbed to the tumor growth. When tumors were needed for other studies, the required number of mice were implanted with the tumor used at the time the serial transplantations were made.

When one of the six tumors mentioned above (BA-1, BA-178, BA-193, C57-220, C57-241 and C3H-263) was transplanted from a mouse of the strain in which it originated into a mouse of a different strain, it was noted that the rate at which the implanted cells multiplied, the size the growth attained and the progress of its regression were, to some extent, characteristic for each tumor. Observations on the characteristic growth and regression of these tumors when transplanted into mice of alien strains led us to conclude that a further study of their growth energy[2] would lead to a clearer understanding of the individuality of malignant growths in general. For this purpose six types of experiments were devised, the results of which are described in the present communication.

1. Series of mice implanted for a study of the rate of growth, size attained by the tumor and the progress of its regression.

2. Series of tumor implantations into alien strain mice at three

2. The term *energy* is used in the sense of inherent power.

stages: (a) while the tumor was still growing, (b) during its regression, and (c) just before it had fully regressed.

3. Attempted serial transplantation of growing tumors into mice of alien strain.

4. Implantation of fragments of a vigorously growing (straight-line) tumor into mice in which a tumor of the same type had previously failed to grow or had grown and regressed.

5. Implantation of grafts from one of the six tumors into mice in which another of the tumors had grown and regressed.

6. Finally, each mouse that had survived the other experiments received a graft from a tumor that originated in a mouse of its own strain.

In the beginning of these studies the implanted mice were examined at intervals of two or three days, and in the majority of them a lump 7x3x3 mm. was palpable on the second or third day. When histological sections of these lumps were prepared it was found that they were vascularized swollen areas of reaction surrounding the small fragment of tumor tissue. In the tumor fragment many cells were undergoing mitosis and a few had begun to invade the adjoining muscle fibers. In most instances the induration subsided by the fifth day, leaving a small, well vascularized sarcoma.

Later in the experiments the growths were measured on the 7th, 9th and 12th day after the implantation and at intervals of 3 days thereafter. The length, width and thickness of each tumor were recorded in millimeters. These measurements were too bulky to use in comparing the sizes of the different tumors, and it was decided to express them in cubic millimeters as a symbol of their comparative volumes. For instance, tumors recorded as 20 mm. long, 10 mm. wide and 5 mm. thick, and as 10x10x10 mm., are listed in the tables as 1000 cu. mm., even though, since the sides of the tumors were rounded, their actual displacement volume was in some instances only a little more than half that figure. Many of the tumors were weighed at the time of transplantation and their actual weights recorded. The unusually large ones weighed as much as 3½ grams, while the smallest weighed less than 0.5 gram. The mice weighed from 20 to 23 grams.[3]

RESULTS

Experiments of the type shown in Table I, devised for a study

3. The large number of deaths that occurred among the mice in these experiments was due to fighting between mice from different stock pens when assembled into series.

TABLE I

THE RATE OF GROWTH, SIZE IN CUBIC MILLIMETERS THE TUMOR ATTAINED AND THE PROGRESS OF REGRESSION OF TUMORS BA-1, BA-178, BA 193, C57-220, C57-241 AND C3H-263 TRANSPLANTED INTO ALIEN STRAIN MICE. [4]

Straight-line tumor used	Transpl. into	Size of resulting tumors on stated days 7	9	12	15	18		
C57 220	A	350	300	128	+	0		
	A	750	1000	250	0	0		
	BA	420	300	200	+	0		
	BA	800	1000	163	0	0		
	C3H	500	224	+	0	0		
	C3H	650	200	+	0	0		
BA 193	A	750	1500	750	125	+	0	
	A	750	245	+	0	0		
	C3H	750	1000	720	+	0		
	C3H	750	1680	1500	175	+	0	
	C57	750	750	600	216	+	0	
	C57	250	250	125	+	0		
BA 178	A	900	800	350	+	0		
	A	1000	760	375	+	0		
	C3H	1500	1525	500	500	350	+	0
	C3H	1500	700	500	500	250	+	0
	C57	1000	250	+	0			
	C57	700	250	+	0			
BA 1	A	1050	1400	1400	375	+	0	
	A	750	1750	750	525	+	0	
	C3H	900	1000	1650	750	+	0	
	C3H	1075	1700	1000	900	245	0	
	C57	525	1500	550	+	0		
	C57	1050	1050	525	+	0		
C57 241	A	1400	1540	750	250	+	0	
	A	1000	1000	750	150	+	0	
	BA	1075	2100	375	+	0		
	BA	1400	1400	840	420	+	0	
	C3H	960	2000	2200	875	375	220	0
	C3H	1450	2100	1750	325	0	0	
C3H 263	A	192	750	2400	1500	750	250	0
	A	750	1000	3000	1260	900	250	0
	BA	875	1100	600	250	+	0	
	BA	750	1000	250	175	+	0	
	C57	1000	1400	500	216	+	0	
	C57	1050	1750	750	+	0		

its regression, were made at the beginning and again, many months of the rate of growth, size attained by the tumor and progress of later, at the close of the study of each of the six tumors. Each series of trans plantations included from two to six mice of each strain and was repeated a number of times for each tumor. The growth energy of the six tumors varied somewhat during the two years of the study.

In the majority of alien-strain hosts the implants of C57-220 tumor grew rapidly, reaching their maximum size on the seventh day. They formed small tumors measuring from 20x10x5 mm. to 10x5x5 mm., which regressed rapidly and in the majority of the hosts disappeared completely within two weeks after the implantation.

The grafts of BA-193 grew rapidly in the alien-strain hosts, the tumors were moderately large on the seventh day, reached their max-

4. d = died. T = transplanted. Capital letters indicate strain of mice. Unless otherwise stated, the tumor volume is listed in cubic millimeters on the 7th, 9th, 12th and later days. Tumor too small for exact measurement are marked + and estimated as 50 cu. mm.

imum size on the 9th to 12th day and, except in a few instances, regressed within 18 days after implantation.

Fragments of BA-178 tumor, when grafted into alien-strain mice, grew rapidly, reached a rather large size within 7 days, and some of them continued to grow until the 9th to 12th day. In the majority of cases the growths regressed rapidly.

BA-1 was the first tumor obtained in this laboratory by means of dibenzanthracene. The early experiments on its transplantability into alien hosts failed to break down its strain specificity and, while grafts grew in alien strain hosts, these growths later regressed (Lewis and Lichtenstein, 1936). The BA-1 tumor has retained the same pathological, cytological and biological features during the course of the 96 generations through which it has been passed. Its inherent power to grow in alien-strain mice has remained unchanged. The pieces implanted in A, C3H and C57 strain mice grew readily, reached a large size by the 9th to 11th day, and then slowly regressed. In some instances the growth remained palpable for three weeks.

C57-241 proved to be the most virulent of the six tumors and in many instances, when grafted into young mice (4-5 weeks old) brought about the death of the host, particularly those of the C3H strain. In older mice grafts of these tumors grew rapidly and reached a large size on the 7th to 9th day. Some of the tumors continued to grow until the 12th or 15th day, while others had undergone considerable regression by that time. Even though these tumors reached a large size the majority of them regressed.

C3H-263 grew to a large size when implanted in mice of alien strains. In many instances the maximum size was not reached until the 12th day or even later. The grafts grew in 100 per cent of the alien strain mice, but with a few exceptions, all the growths regressed

In studies on the transplantability of tumors the age of the mouse played a considerable part in its survival. In animals 4 to 5 weeks old tumor grafts grew much larger in proportion to the size of the host than they did in older animals. Many of the delicate young mice did not survive until the tumor regressed. Mice 8 to 10 weeks old were more favorable for the experiments. These mice were almost full grown when implanted, so that a tumor 25x10x10 mm. was readily borne. Unless the tumors grew much larger than that before regression began, the hosts survived. Regardless of the age of the host, how-

TABLE II

SIZE OF TUMORS IN SECOND GENERATION IN ALIEN STRAIN MICE. 1. FROM GRAFTS OF TUMORS THAT WERE GROWING PROGRESSIVELY. [4]

Tumor	Growing Progressively in				No. of Days	Trans. Into	Size of Growth in 2nd Generation						
BA 1	A	750	1500	3000	11	A	1400	735	+	0			
						C3H	1400	980	2000	T			
						C57	1540	1050	+	0			
BA 1	C3H	750	1400	2300	11	A	1540	700	+	0			
						C3H	175	175	0	0			
						C57	450	750	3675	T			
BA 1	C57	700	1400	2000	11	A	200	200	+	0			
						C3H	250	525	105	0			
						C57	90	+	0				
BA 178	A	250	1080		9	A	+	0	0				
						C3H	0	d					
						C57	0	d					
BA 178	C3H	350	900		9	A	+	0	0				
						C3H	+	0	0				
						C57	+	0	0				
BA 178	C57	125	750		9	A	+	0	0				
						C3H	+	0	0				
						C57	+	0	0				
BA 193	C3H	750	4800	9900	42	A	1050	1050	+	0			
						C3H	7750	1000	125	0			
						C57	50	750	+	0			
BA 193	A	700	1920	2400	46	A	125	+	0				
						C3H	175	+	0				
						C57	200	+	0				
BA 193	C3H	750	3000	9900	49	A	1000	1000	175	0			
						C3H	500	500	300	+	0		
						C57	500	500	350	+	0		
C57 220	A	750	1000		9	A	0	0	0				
						BA	0	0	0				
						C3H	0	0	0				
C57 220	BA	800	1500		9	A	0	0	0				
						BA	0	0	0				
						C3H	0	0	0				
C57 220	C3H	700			7	A	125	+	0				
						BA	63	+	0				
						C3H	72	+	0				
C57 241	A	1000			8	A	900	900	+	0			
						BA	175	175	400	+	0		
						C3H	250	432	+	500	3000	4500	T
C57 241	BA	1000			8	A	375	375	117	0			
						BA	125	27	0				
						C3H	600	750	280	+	0		
C57 241	C3H	1000			8	A	840	750	280	+	0		
						BA	250	d					
						C3H	1200	1200	375	320	0		
C57 241	C3H	250	2000	3750	11	A	250	250	+	0			
						BA	125	125	125	0			
						C3H	125	250	750	1000	2000	3750	T
C3H 263	A	750			8	A	250	250	+	+	0		
						BA	63	63	0				
						C57	90	525	+	0			
C3H 263	A	750	3000	9900	35	A	125	300	100	+	0		
						BA	125	2000	5625	d			
						C57	375	2000	T				
C3H 263	BA	1000			8	A	250	1000	500	350	1800	2400	T
						BA	150	600	600	600			
						C57	280	800	800	216	0		
C3H 263	BA	750	1400	2700	27	A	125	230	500	1050	1260	2000	T
						BA	125	216	343	1050	1500	T	
						C57	400	32	0				
C3H 263	C57	1400			8	A	351	525	125	+	0		
						BA	225	720	125	+	0		
						C57	375	900	900	T			

4. d = died. T = transplanted. Capital letters indicate strain of mice. Unless otherwise stated, the tumor volume is listed in cubic millimeters on the 7th, 9th, 12th and later days. Tumor too small for exact measurement are marked + and estimated as 50 cu. mm.

2. From Grafts of Tumors Whose Growth Was Stationary

Tumor	Stationary in			No. of Days	Trans. Into	Size of Tumor in 2nd Generation						
BA 1	C57	750	1500	1500	11	A	0	0	0	0			
						C3H	0	0	0	0			
						C57	0	0	0	0			
BA 178	A	1050	1400	1400	13	A	175	175	+	0			
						C3H	+	0	0	0			
						C57	125	0	0	0			
C57 241	C3H	500	2000	2000	19	A	175	175	350	280	+	0	
						BA	250	250	45	0	0	0	
						C3H	400	600	600	280	125	0	
C3H 263	BA	525	1100	1100	15	A	175	175	250	250	400	1050	T
						BA	64	0	0				
						C57	250	1500	2000	3750	T		
C3H 263	A	1400	1932	1932	11	A	+	0	0	0	0	0	
						BA	+	0	0	0	0	0	
						C57	+	0	0	0	0	0	
C3H 263	BA	550	750	750	18	A	300	0	0	0	0	0	
						BA	63	0	0	0	0	0	
						C57	175	0	0	0	0	0	
C3H 263	C57	350	900	900	11	A	63	0	0	0	0	0	
						BA	63	0	0	0	0	0	
						C57	+	0	0	0	0	0	
C3H 263	C57	250	750	750	21	A	0	0	0	0	0	0	
						BA	0	0	0	0	0	0	
						C57	0	0	0	0	0	0	
C3H 263	C57	375	3750	3750	40	A	0	0	0	0	0	0	
						BA	0	0	0	0	0	0	
						C57	0	0	0	0	0	0	

3. From Grafts of Tumors Whose Growth Was Regressing.

Tumor	Regressing in			No. of Days	Trans. Into	Size of Tumor in 2nd Generation				
BA 1	A	750	1050	750	16	A	0	0	0	0	
						BA	+	600	2500	3000	d
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 1	C3H	900	1000	630	15	A	0	0	0	0	
						BA	+	600	3750	4500	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 1	C57	525	735	250	11	A	0	0	0	0	
						BA	0	+	1400	3300	d
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 178	A	600	90		12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 178	C3H	900	600		12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 178	C57	600	75		12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 193	A	900	420		12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 193	C3H	125	250	125	12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	
BA 193	C57	125	245	125	12	A	0	0	0	0	
						C3H	0	0	0	0	
						C57	0	0	0	0	

TABLE II (Cont'd)

Tumor	Stationary in			No. of Days	Trans. into	Size of Tumor in 2nd Generation				
C57 220	A	350	250 128	10	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	0	0	0	0	
C57 220	BA	420	300 250	10	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	0	0	0	0	
C57 220	C3H	250	120	9	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	0	0	0	0	
C57 241	A	1400	1540 250	15	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	0	0	0	0	
C57 241	BA	375	1400 720	15	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	0	0	0	0	
					C57	250	750	1500	2500	d
C57 241	C3H	1750	4500 1540	15	A	+	0	0	0	
					BA	+	0	0	0	
					C3H	+	0	0	0	
C3H 263	A	175	900 150	15	A	0	0	0	0	
					BA	0	0	0	0	
					C57	0	0	0	0	
C3H 263	BA	375	600 128	18	A	0	0	0	0	
					BA	0	0	0	0	
					C57	0	0	0	0	
C3H 263	C57	150	750 600	13	A	0	0	0	0	
					BA	0	0	0	0	
					C3H	350	840	2000	3000	d
					C57	0	0	0	0	

4. FROM GRAFTS OF TUMORS THAT HAD REGRESSED.

Tumor	Regressing in			No. of Days	Trans. Into	Size of Tumor in 2nd Generation				
BA 1	A	980	1400 +	18	BA	0	0	0	0	
BA 1	C3H	375	1260 +	18	BA	0	0	0	0	
BA 178	A	750	90 45	14	BA	0	0	0	0	
BA 178	C3H	250	175 +	14	BA	0	0	0	0	
BA 178	C57	+	125 +	12	BA	0	0	0	0	
BA 193	A	125	500 45	13	BA	0	0	0	0	
BA 193	C3H	125	280 64	12	BA	0	0	0	0	
BA 193	C57	125	180 27	12	BA	0	0	0	0	
C57 220	BA	750	250 125	10	A	0	0	0	0	
					BA	0	0	0	0	
					C57	+	250	1200	1500	d
C57 220	A	200	200 175	10	C57	0	+	250	2500	d
C57 220	A	600	125	10	A	0	0	0	0	
					C57	+	175	350	750	1500 d
C57 220	C3H	175	+ +	10	C57	0	0	0	0	
C57 241	A	1000	1000 90	18	C57	0	0	0	0	
C57 241	BA	375	1000 +	18	C57	0	0	0	0	
C57 241	C3H	250	1000 43	18	C57	0	0	0	0	
C3H 263	A	480	900 +	27	C3H	0	0	0	0	
C3H 263	BA	375	600 28	18	C3H	0	0	0	0	
C3H 263	C57	250	750 45	13	C3H	0	0	0	0	

ever, mice of the C3H strain were more favorable for the growth of these six tumors than mice of the other three strains, and more frequently succumbed to the tumors.

Before describing the other five types of experiments employed in these studies it is important to emphasize the fact that every one of the 294 *straight-line* tumors, carried through many serial generations in this laboratory, proved to be transplantable. Only 4 of the many thousands of pure inbred mice used failed to develop a tumor following its implantation with a graft of one of the *straight-line* tumors of the same strain. On the other hand, with the exception of the regressing tumors, the grafts of the 294 dibenzanthracene-induced tumors did not grow to what could be termed tumor size when implanted into alien strain mice.

Experiment II.

In this experiment further information regarding the inherent growing power of these six tumors was sought by studying certain intrinsic qualities of the growths that reached a moderately large size in alien-strain hosts. For this purpose tumors that were still growing, others that were regressing, and those that had almost completely regressed were transplanted into mice of A, BA, C3H and C57 strains.

As shown in Table II, which gives the results obtained in one experiment of this type with each tumor, grafts of the BA-178, BA-193 and C57-220 tumors, with only a few exceptions, failed to grow in the second generation in alien strain hosts. Grafts of BA-1, C57-241 and C3H-263 did or did not grow in alien strain hosts according to the stage reached by the tumor at the time of transplantation. Twenty-one tumors were transplanted while still growing. Of these the grafts of one BA-1 in a C57 strain mouse, three BA-178 in A, C3H and C57 strain hosts failed to grow in the alien strain mice in the second generation.

Eighteen tumors were transplanted after they had begun to regress and not one graft of these tumors grew in an alien strain mouse. On the other hand, grafts of these tumors did grow in mice of the strain in which the tumor had originated.

Eleven tumors were transplanted at a time when their growth was recorded as stationary. Grafts of 4 of them grew in alien strain mice, while grafts of the other seven failed to do so. It may be that four of the growths recorded as stationary were actually larger and the

other seven smaller than when measured two or three days earlier.

Eighteen tumors were transplanted when they had almost entirely regressed. Three of them were still a measurable size, one 7x5x5, the other two 5x5x5 mm. Grafts of these tumors grew in mice of the strain of their origin but not in mice of alien strains. Grafts of the smaller tumors failed to grow in every one of the implanted mice, even in those of the strain of their origin.

Experiment III.

The tumors that showed progressive growth in the second generation were transplanted through as many generations as possible in alien strains. In this way three lines of C3H-263 (Chart I) and two lines of C57-241 tumors (Chart II) were passed through more than five generations in alien strain mice. By the time the C57-241 tumors had

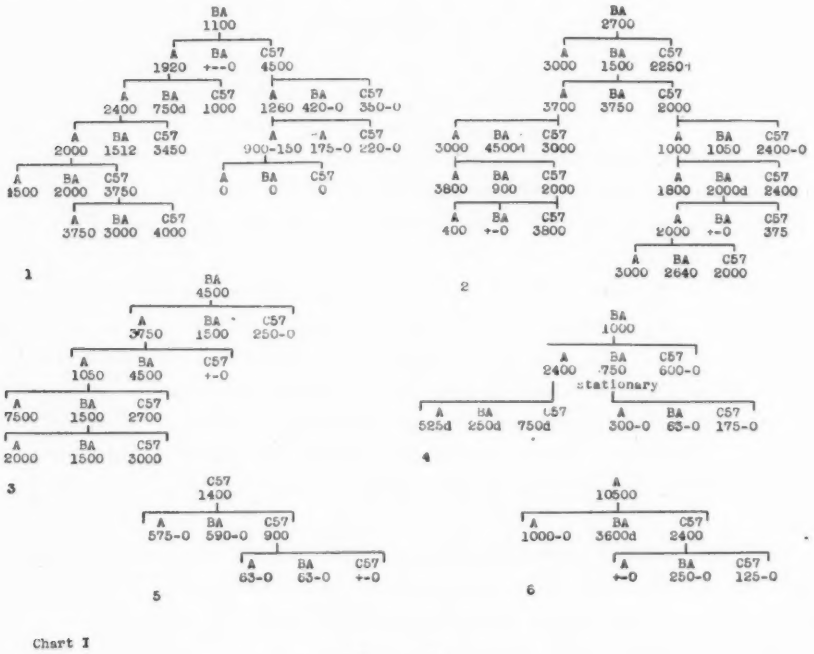

CHART I.

Serial transplantation and size attained in cu. mm. of six C3H-263 tumors in alien strain mice.

Figures 1, 2 and 3 show three C3H-263 tumors that were passed through 6, 7 and 5 generations respectively. Figures 4, 5, and 6 show attempts to transplant three other C3H-263 tumors. Each regressed in the 3rd generation.

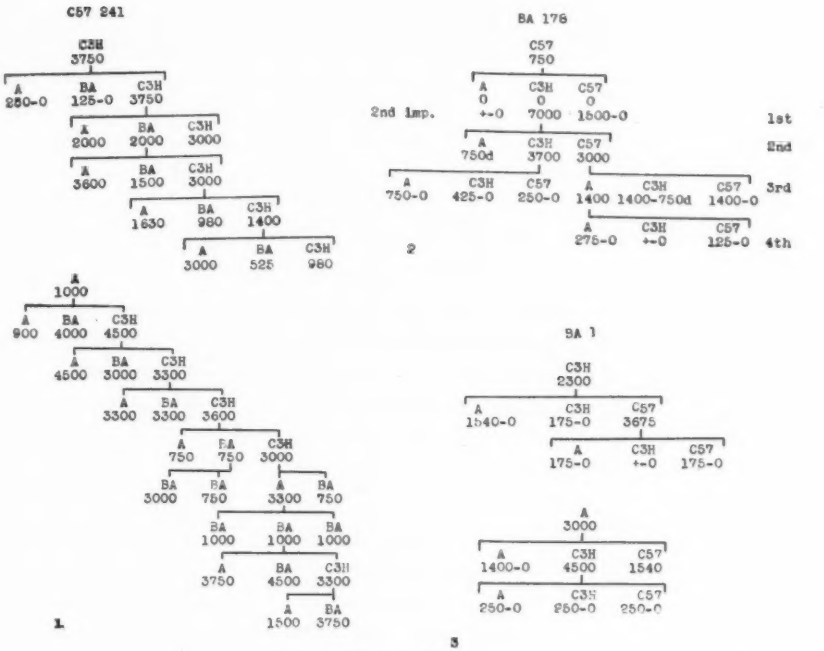

CHART II.

Serial transplantation and size attained in cu. mm. of tumors C57-241, BA-178 and BA-1 in alien strain mice.

Figure 1 shows the passage of two C57-241 tumors, one through 6 and the other through 9 generations.

Figure 2 shows the passage of a BA-178 tumor that grew from a second implantation of the tumor in a C3H strain mouse. The tumors regressed in the 4th generation.

Figure 3 shows attempts to transplant two BA-1 tumors in alien strain mice. Each regressed in the 3rd generation.

passed through four or five generations they had become transplantable into alien strain mice; that is, they grew equally well in mice of each of the alien strains and could thereafter be transplanted into as few as two mice of any strain at each serial passage. One of these tumors was passed in this manner through 15 serial transplantations. The growth of the C3H-263 tumors proved to be more unstable. While the grafts grew to a moderately large size in one alien strain host in each generation, in some instances the tumors regressed or failed to attain a moderate size in the other two alien strain hosts. Three other attempts at serial transplantation of C3H-263 tumors were made (Chart I). All of them failed after the third generation.

In the serial transplantation of tumor C57-241, quite unintentionally the tumors growing in the C3H strain hosts were transplanted more frequently than those in other strains. However, as may be seen from Chart II, the favorable growth of the C57-241 tumor was not dependent upon the strain of its host. On the other hand, grafts of tumor C3H-263 grew to a transplantable size more frequently in mice of A and C57 strains than in those of BA strain.

One BA-178 tumor (Chart II) was passed through three generations in alien strain mice, but the tumors regressed in the fourth generation. Two BA-1 tumors were transplanted through two generations in alien strain mice, but the growths were small and regressed in the third generation. Three BA-193 tumors, which grew progressively in alien strain hosts, were transplanted into normal A, C3H and C57 strain mice. The resulting tumors regressed in the second generation. Not one C57-220 tumor graft grew larger than 5x5x5 mm. in the second generation in alien strain mice.

The attempt at serial transplantation of the six regressing tumors into mice of alien strains demonstrated the marked differences in the growth energy of these tumors. That of C57-220 was not sufficient to carry it into a second generation. The growth energy of BA-193 resulted in tumors in the second generation in only three instances. Although each of these tumors attained a rather large size they regressed before they could be transplanted. In one instance a BA-178 tumor that grew to a large size in the second generation was transplanted through another generation, but regression of the growths occurred in the fourth generation. BA-1 was successfully transplanted through two generations but the tumors in the third generation were smaller and soon regressed. On the other hand, the growth energy of C57-241 and C3H-263 was so great that in a number of instances it resulted in the serial transplantation of these two tumors through more than five generations in alien strain hosts.

In the attempts at serial transplantation it was found that the strain from which the tumor was transplanted did not necessarily prove to be more favorable than the other two alien strains for the growth of that tumor in the succeeding generation.

Experiment IV.

Many mice free from tumor accumulated from the series used in Experiment II. In some of these a tumor fragment had grown and

TABLE III

SIZE ATTAINED BY GRAFTS OF STRAIGHT-LINE TUMORS IN ALIEN STRAIN MICE IN WHICH A PREVIOUS GRAFT OF THE SAME TYPE OF TUMOR FROM AN ALIEN STRAIN HOST HAD GROWN AND REGRESSED. [4]

Mice of Strain	Grafted with Tumor	from	Size of Growth in Days 7	9	12	15	24	Grafted with Tumor		Size of Growth in Days 7	9	12	15	20
A	1	A	1400	735	+	0	0	BA	1	125	0	0	0	0
A	1	C3H	1540	700	+	0	0	BA	1	+	0	0	0	0
A	1	C57	175	+	0	0	0	BA	1	+	0	0	0	0
A	1	C3H	250	175	+	0	0	BA	1	+	0	0	0	0
A	1	C57	250	250	+	0	0	BA	1	+	0	0	0	0
C3H	1	C3H	250	0	0	0	0	EA	1	+	0	0	0	0
C3H	1	C57	250	525	105	0	0	BA	1	0	0	0	0	0
C57	1	A	1050	1050	+	0	0	BA	1	0	0	0	0	0
C57	1	C57	175	+	0	0	0	BA	1	0	0	0	0	0
C57	1	C3H	250	+	0	0	0	BA	1	0	0	0	0	0
C57	1	C57	90	+	0	0	0	BA	1	0	0	0	0	0
A	220	C57	250	175	+	0	0	C57	220	125	0	0	0	0
A	220	C57	125	125	27	0	0	C57	220	+	0	0	0	0
A	220	C57	250	175	27	0	0	C57	220	125	0	0	0	0
A	220	C57	375	175	0	0	0	C57	220	+	0	0	0	0
A	220	C57	250	175	0	0	0	C57	220	+	0	0	0	0
BA	220	C57	250	175	+	0	0	C57	220	+	0	0	0	0
BA	220	C57	250	175	0	0	0	C57	220	+	0	0	0	0
BA	220	C57	525	1500	1500	125	0	C57	220	+	0	0	0	0
BA	220	C57	375	200	175	0	0	C57	220	+	0	0	0	0
C3H	220	C57	250	175	+	0	0	C57	220	+	0	0	0	0
C3H	220	C57	250	175	+	0	0	C57	220	+	0	0	0	0
C3H	220	C57	735	175	27	0	0	C57	220	+	0	0	0	0
C3H	220	C57	1120	840	+	0	0	C57	220	+	0	0	0	0
C3H	220	C57	175	175	250	0	0	C57	220	+	0	0	0	0
A	241	BA	250	250	+	0	0	C57	241	0	0	0	0	0
A	241	BA	375	375	175	+	0	C57	241	+	0	0	0	0
A	241	C3H	175	175	350	280	0	C57	241	+	0	0	0	0
A	241	C3H	250	250	+	0	0	C57	241	+	0	0	0	0
A	241	A	600	750	175	+	0	C57	241	0	0	0	0	0
BA	241	BA	125	27	27	+	0	C57	241	+	0	0	0	0
BA	241	C3H	250	250	250	250	0	C57	241	+	0	0	0	0
BA	241	C3H	125	125	125	0	0	C57	241	+	0	0	0	0
BA	241	BA	350	125	27	0	0	C57	241	+	0	0	0	0
C3H	241	BA	600	750	280	0	0	C57	241	+	0	0	0	0
C3H	241	C3H	400	600	690	125	0	C57	241	+	0	0	0	0
A	263	A	250	250	0	0	0	C3H	263	+	0	0	0	0
A	263	BA	300	+	0	0	0	C3H	263	0	0	0	0	0
BA	263	C57	225	720	125	+	0	C3H	263	+	0	0	0	0
BA	263	C57	250	420	250	175	0	C3H	263	+	0	0	0	0
C57	263	C57	350	350	350	175	0	C3H	263	+	0	0	0	0
C57	263	BA	280	800	800	216	0	C3H	263	+	0	0	0	0
C57	263	BA	175	0	0	0	0	C3H	263	0	0	0	0	0
C57	632	A	90	525	+	0	0	C3H	263	+	0	0	0	0

regressed, in others the graft had failed to grow. For the purpose of testing whether these mice were resistant to the growth of the particular tumor they received, or had become so, they were implanted 3 to 5 weeks later with fragments of a tumor of the same kind. In order to be certain that the grafts were from vigorously growing tumors, straight-line tumors were used for this second transplantation.

Tables III and IV show the size to which the first graft had grown and also the size attained by the graft of the straight-line tumor implanted in the mouse some weeks later. As may be seen in these tables, in all but three cases, the second tumor graft failed to grow in the 46

4. d = died. T = transplanted. Capital letters indicate strain of mice. Unless otherwise stated, the tumor volume is listed in cubic millimeters on the 7th, 9th, 12th and later days. Tumor too small for exact measurement are marked + and estimated as 50 cu. mm.

TABLE IV

SIZE ATTAINED BY GRAFTS OF STRAIGHT LINE TUMORS IN ALIEN STRAIN MICE IN WHICH A PREVIOUS GRAFT OF THE SAME TYPE OF TUMOR FROM AN ALIEN STRAIN HOST HAD FAILED TO GROW. [4]

Mice of Strain	Grafted with Tumor	from	Size of Growth on Stated Days 7	9	12	15	Grafted with Tumor		Size of Growth on Stated Days 7	9	12	15	21
A	1	C57	0	0	0	0	BA	1	245	500	500	500	0
A	1	C3H	0	0	0	0	BA	1	750	350	+	0	0
A	1	A	0	0	0	0	BA	1	+	0	0	0.	0
A	1	C57	+	+	0	0	BA	1	0	0	0	0	0
A	1	C57	0	0	0	0	BA	1	+	0	0	0	0
C3H	1	C57	0	0	0	0	BA	1	750	3000	3000	3000	3000
C3H	1	C3H	+	0	0	0	BA	1	175	125	0	0	0
C3H	1	A	0	0	0	0	BA	1	525	1050	1050	3000	d
C3H	1	C57	+	0	0	0	BA	1	375	1050	1050		
C3H	1	C57	0	0	0	0	BA	1	63	0	0		
C3H	1	C3H	125	0	0	0	BA	1	250	350	700	300	3750
C57	1	C57	0	0	0	0	BA	1	750	490	125	0	0
C57	1	C3H	0	0	0	0	BA	1	750	490	490	125	0
C57	1	A	0	0	0	0	BA	1	490	1500	1500	2250	3000d
C57	1	C57	0	0	0	0	BA	1	200	0	0	0	
C57	1	C57	90	0	0	0	BA	1	175	175	0		
BA	1	A	0	0	0	0	BA	1	420	1500	2000	4950	
BA	1	C3H	0	0	0	0	BA	1	420	1500	1800	3000	
BA	1	C57	0	0	0	0	BA	1	375	1400	3750	4500	
A	178	A	+	0	0	0	BA	178	250	750	1000	d	
A	178	A	0	0	0	0	BA	178	600	250	+	0	
A	178	A	+	0	0	0	BA	178	750	250	+	0	
A	178	C57	+	+	0	0	BA	178	+	+	0	0	
A	178	A	0	0	0	0	BA	178	3000	3000	4000	4000	1000
A	178	C3H	0	0	0	0	BA	178	2250	2250	500	125	0
A	178	C57	0	0	0	0	BA	178	1500	2250	2250	750	500
C3H	178	C3H	+	0	0	0	BA	178	3000	3000	3000	3000	3000
C3H	178	C57	+	0	0	0	BA	178	1600	1600	1600	3750	7000T
C3H	178	C3H	0	0	0	0	BA	178	2250	3000	3000	3000	1500
C3H	178	C57	+	0	0	0	BA	178	3000	2250	2400	3000	900
C57	178	C3H	+	0	0	0	BA	178	1500	1500	3000	3000	1500
C57	178	C57	+	0	0	0	BA	178	1500	1500	500	+	0
C57	178	A	0	0	0	0	BA	178	1500	1200	125	+	0
C57	178	C3H	+	0	0	0	BA	178	1500	1500	250	125	0
C57	178	C57	+	0	0	0	BA	178	750	1500	2700	3000	1500
BA	178	A	0	0	0	0	BA	178	800	1800	3000		
BA	178	A	0	0	0	0	BA	178	1500	2160	3000		
BA	178	C3H	0	0	0	0	BA	178	250	350	750		
BA	178	C3H	0	0	0	0	BA	178	375	750	750		
BA	178	C57	0	0	0	0	BA	178	400	400	700		
BA	178	C57	0	0	0	0	BA	178	750	750	750		
A	193	A	+	0	0	0	BA	193	250	d			
A	193	C57	+	0	0	0	BA	193	+	+	0	0	
A	193	C3H	+	0	0	0	BA	193	+	+	0	0	
A	193	C57	+	0	0	0	BA	193	125	+	0	0	
A	193	A	+	0	0	0	BA	193	+	0	0	0	
C3H	193	A	+	0	0	0	BA	193	375	375	175	45	
C3H	193	C3H	+	0	0	0	BA	193	250	500	250	175	
C3H	193	C57	+	0	0	0	BA	193	+	0	0	0	
C3H	193	C57	+	0	0	0	BA	193	125	+	0	0	
C3H	193	A	+	0	0	0	BA	193	500	1100	1440	500	
C57	193	A	+	0	0	0	BA	193	+	0	0	0	
C57	193	C3H	+	0	0	0	BA	193	+	0	0	0	
C57	193	C57	+	0	0	0	BA	193	+	0	0	0	
C57	193	C3H	+	0	0	0	BA	193	+	0	0	0	
C57	193	C57	+	0	0	0	BA	193	+	0	0	0	
C57	193	A	+	0	0	0	BA	193	+	0	0	0	
BA	193	A	0	0	0	0	BA	193	750	750	900	1500	2000
BA	193	C3H	0	0	0	0	BA	193	250	500	1500	3000	6000
BA	193	C57	0	0	0	0	BA	193	150	500	1500	3000	5600
A	220	A	+	0	0	0	C57	220	250	175	+	0	
A	220	BA	+	0	0	0	C57	220	120	27	0	0	
A	220	C3H	+	0	0	0	C57	220	250	175	+	0	
A	220	A	+	0	0	0	C57	220	250	175	27	0	
A	220	BA	+	0	0	0	C57	220	375	175	+	0	
BA	220	A	+	0	0	0	C57	220	250	175	+	0	
BA	220	BA	+	0	0	0	C57	220	250	175	+	00	
BA	220	C3H	+	0	0	0	C57	220	175	+	0	0	125
BA	220	A	+	0	0	0	C57	220	525	525	525	15 0	
BA	220	BA	+	0	0	0	C57	220	375	200	+	0	
BA	220	C3H	63	0	0	0	C57	220	+	0	0	0	
C3H	220	A	+	0	0	0	C57	220	250	175	+	0	
C3H	220	BA	+	0	0	0	C57	220	250	175	+	0	
C3H	220	C3H	+	0	0	0	C57	220	175	175	250	0	
C3H	220	A	+	0	0	0	C57	220	735	175	27	0	
C3H	220	BA	+	0	0	0	C57	220	1120	840	+	0	
C3H	220	C3H	72	0	0	0	C57	220	+	0	0	0	
C57	220	A	0	0	0	0	C57	220	250	1750	3750		
C57	220	BA	0	0	0	0	C57	220	400	1000	2000		

4. d = died. T = transplanted. Capital letters indicate strain of mice. Unless otherwise stated, the tumor volume is listed in cubic millimeters on the 7th, 9th, 12th and later days. Tumor too small for exact measurement are marked + and estimated as 50 cu. mm.

TABLE IV (Cont'd)

Mice of Strain	Grafted with Tumor	from	Size of Growth on Stated Days 7	9	12	15	Grafted with Tumor	Size of Growth on Stated Days 7	9	12	15	21
A	241	C3H	+	+	0	0	C57 241	0	0	0	0	
A	241	A	0	0	0	0	C57 241	250	+	0	0	
A	241	BA	0	0	0	0	C57 241	90	+	0	0	
BA	241	A	0	0	0	0	C57 241	+	105	0	0	
BA	241	BA	0	0	0	0	C57 241	150	750	375	+	
C3H	241	A	0	0	0	0	C57 241	1250	750	125	+	
C3H	241	BA	0	0	0	0	C57 241	180	+	0	0	
C57	241	A	0	0	0	0	C57 241	375	1500	2500		
C57	241	BA	0	0	0	0	C57 241	750	1400	2200	3750	
C57	241	A	0	0	0	0	C57 241	525	2000	2500		
A	263	C57	+	0	0	0	C3H 263	750	1000	3000	3750	3000
BA	263	A	+	0	0	0	C3H 263	250	750	1500	2000	3300
BA	263	C57	+	0	0	0	C3H 263	63	0	0	0	0
BA	263	C57	+	0	0	0	C3H 263	375	750	1920	1920	2640
BA	263	BA	64	0	0	0	C3H 263	216	360	125	+	0
BA	263	BA	63	0	0	0	C3H 263	0	0	0	0	0
BA	263	BA	+	0	0	0	C3H 263	250	750	1500	2200	3300
C57	263	C57	+	0	0	0	C3H 263	3000	1050	700	1050	1932
C57	263	A	+	0	0	0	C3H 263	750	750	175	+	0
C57	263	C57	+	0	0	0	C3H 263	250	+	0	0	0
C57	263	C57	+	0	0	0	C3H 263	375	525	+	+	0
C3H	263	A	0	0	0	0	C3H 263	750	1000	3000	died	
C3H	263	BA	0	0	0	0	C3H 263	520	1800	3600	died	
C3H	263	C57	0	0	0	0	C3H 263	750	3000	3750	died	

mice in which a previous graft of that tumor had grown and regressed (Table III), while in 82 of the 102 mice in which the first graft of a tumor failed to grow (Table IV), the graft of the straight-line tumor of that kind grew to a measurable size. In each of the four instances (3 in Table III and 1 in Table IV) in which the second graft grew in a mouse in which a previous graft of that tumor had grown, either the first or the second growth was small and may have been a lump due to reaction rather than to tumor-cell multiplication. In some experiments the mice in which a graft of tumor had grown and regressed were implanted with three successive grafts of the same type of tumor a week or ten days apart. Not one of these later grafts developed into a tumor. These results show that the mice in which a graft of tumor failed to grow were not resistant to the growth of the cells of that tumor, while those in which a tumor had grown and regressed had developed a resistance to the growth of another graft of that tumor.

The results shown in Table IV indicate that the development of resistance to a second graft of tumor is not dependent upon the size of the tumor resulting from the first graft, provided it reached a measurable size before regression began. In these experiments mice in which tumors had grown no larger than 10x5x5 mm. or 250 cu. mm. proved to be resistant to the growth of a second implant of that tumor as well as those in which the tumor from the first implant reached a size of 20x10x10 mm. or 2000 cu. mm. before regression occurred.

TABLE V

A FEW EXAMPLES OF THE SUCCESSIVE GROWTH OF MORE THAN ONE OF THE SIX TUMORS IN ALIEN STRAIN HOSTS. [4]

Mouse	Graft	Tumor	Size of Tumors on Stated Days									
			7	9	12	15	18	21	24	27	30	36
A 65	Dec. 15	BA 178	3000	4000	4000	4000	1000	1000		175	125	+
	Jan. 10	C3H 263	105	525	700	700	died					
C57 34	Jan. 6	BA 178	1000	250	+	0						
	Jan. 19	C3H 263	+	+	0	0						
	Feb. 7	BA 1	+	672		1505	2750	3000	4500	7500	died	
A 20	Dec. 20	BA 193	500	1500	1500	125	+	0				
	Jan. 6	BA 178	500	1050	1500	1500	588	350	175	+	0	
	Feb. 11	C3H 263	500	600	980	1260	1800	3375	6000	7200	died	
C3H 1	Dec. 1	BA 193	750		1680		1800	1500	175	0		
	Dec. 30	BA 178	750	3000	3960		7500	7500	died			
C3H 6	Dec. 1	BA 193	750		750	+	0					
	Dec. 30	BA 178	500	245	+	0	0					
	Jan. 23	C57 241	750	1608		3750	9000	9000	died			
C3H 20	Dec. 20	BA 193	750	1000	720	+	0					
	Jan. 6	BA 178	64	+	0	0						
	Jan. 23	C57 241	750	3000		8400		died				
C57 21	Dec. 20	BA 193	750	750	600	216	+	0				
	Jan. 6	BA 178	+	0	0	0	0					
	Feb. 11	C3H 263	420	2000	2000	2000	2000	375	175	0		
C3H 91	Dec. 14	C57 220	1120	840	+	0						
	Jan. 20	C57 220	0	0	0	0	0					
	Feb. 6	C57 241	+	+	+	350	735	2000	3000	4320	died	
C3H 90	Dec. 14	C57 220	735	175	27	0						
	Jan. 20	C57 220	0	0	0	0						
	Feb. 6	C57 241	+	+	125	175	250	350	750	1428	2000	died
C3H 111	Jan. 12	C3H 193	750	1000	1000	125	+	0				
	Feb. 24	BA 1	525	420	175	+	0					
	Mar. 16	BA 178	64	1200	1500	1800	died					
A 23	Sept. 16	C57 220	500	750	700	700	250	+	0			
	Sept. 19	C57 241	350	1000	1500	750	125	0	0			
	Oct. 21	C3H 263	375	1500	1500	750	250	0				
	Nov. 18	BA 178	750	500	250	+	0					

Experiment V.

In order to ascertain whether mice that had become resistant to the growth of a given tumor were immune from the growth of tumor tissue in general, a number of them were implanted with fragments from another one of the six tumors. In Table V are shown a few of the many instances in which two or more of the six tumors grew and regressed in a single host.

These results demonstrate that these six tumors, although pathologically alike, are biologically so different that, while the growth and regression of one of them brought about in the host a resistance to the growth of a subsequent graft of the same kind of tumor, it did not bring about a resistance to grafts from any of the other five regressing tumors, even when the two tumors originated in mice of the same strain, as for instance BA-1 and BA-178 or C57-220 and C57-241. In a few instances a graft of a third BA tumor grew in a mouse in which each of the other BA tumors had grown and regressed, as is shown in

C3H III, Table V. In other words, the resistance developed in the host was against the growth of particular tumor cells, not of cells of a particular strain.

Experiment VI.

At the close of these studies, the mice that had survived the foregoing experiments were assembled and each one was engrafted with fragments of a tumor that had originated in a mouse of its own strain. Not one mouse of the BA, C3H or C57 strains proved to be resistant to the growth of a graft of the straight-line tumor (1, 178, 193, 220, 241 and 263) which originated in its own strain. While in some instances the growth of the graft was somewhat delayed, the implants eventually developed into large tumors.

DISCUSSION

At the time the cells become malignant, they apparently take on certain peculiarities, such as type of nucleoli, amount of mitochondria, type of mitosis and consistency of cytoplasm and cell wall (W. H. Lewis, 1935, 1939[1 2]). These cytological features, combined with the histological nature of the cells, establish certain cancer types that can be identified by pathological studies and by studies of the living cells in tissue cultures. In addition to the cytological and pathological characteristics of malignant cells, certain biological features, such as influence upon the blood-forming organs of the host, degree of strain specificity, growth energy and individuality of the tumor cells, also seem to be taken on by the cells at the time malignancy is assumed, for each of the primary induced tumors that exhibited peculiar biolocal characteristics has retained them throughout its serial transplantation. This is rather amazing when we consider the great number of cell multiplications required for a tiny graft (1 cu. mm.) of a tumor such as BA-1 to grow into a tumor 3000 to 4500 cu. mm. in each generation of the 96 serial transplantations that have taken place during the $3\frac{1}{2}$ years this tumor has been studied. Nevertheless, the BA-1 tumors studied today exhibit the cytological features, the same degree of myeloid stimulating quality and the ability to grow and regress in alien strain hosts that characterized this tumor when it was first examined.

The biological peculiarities characteristic of individual tumors have been retained by certain malignant cells through prolonged cultivation

in heterotrophic media in tissue cultures and through freezing at −70°C for several months.

The individuality of tumor cells is difficult to demonstrate in studies of the majority of tumors, because they either grow or fail to grow when transplanted. Certain of the rat and mouse tumors do undergo growth and regression in a percentage of the implanted hosts. It is not possible, however, to select for experimental purposes the grafts that are likely to regress. For this reason the six spindle-celled sarcomata BA-1, BA-178, BA-193, C57-220, C57-241 and C3H-263, which grow to a certain extent in mice of any of the available strains and rarely fail to regress in hosts of an alien strain, are particularly favorable for a study of certain biological characteristics of tumor cells.

As was stated by Strong (Lewis and Strong, 1934) "Biologists recognize that both extrinsic and intrinsic factors are involved in all living phenomena." In these transplantation studies it was necessary to consider as intrinsic factors the strain of mouse in which the tumor originated (that is, the strain of the tumor cells), the growth energy of the particular tumor, the individuality of the tumor cells, and whether the tumor was growing progressively or regressing at the time it was transplanted. As extrinsic factors it was necessary to consider the strain of the host into which the tumor was implanted, the age of the host, and whether a previous graft of that tumor had grown and regressed in the host.

Each of the six tumors exhibited a characteristic growth energy; that is, each tumor when transplanted into alien strain mice grew to a characteristic size within a given number of days, and the number of generations through which these tumors could be serially transplanted into mice of an alien strain was distinctive for each tumor. Each of the six tumors exhibited a cell individuality; that is, the growth and regression of a tumor rendered the host resistant to a later graft of that tumor, but not to a graft from one of the other five, even though the two tumors originated in mice of the strain. On the other hand, each of the 6 tumors retained its strain specificity, in that grafts of them grew progressively in every mouse of the strain of the tumor's origin, regardless of whether implants of one or more tumors originating in mice of other strains had failed to grow or had grown and regressed in these mice.

Just why the spindle cells of these tumors should exhibit biological

individuality is at present a matter of surmise. While the cells of tumors 1, 178 and 193 resemble one another and the three tumors originated in the same manner in BA strain mice, it is conceivable that the spindle-cells that became malignant to form each tumor arose from a different tissue. On the other hand, the geneticist might interpret the individuality of the spindle cells as dependent upon the genes affected at the time the influence that resulted in malignancy disturbed the normal arrangement of the genes in the chromosomes of those particular cells; while investigators interested in pure inbred strains of animals might attribute the individuality of the cells of tumors arising in mice of the same pure inbred strains to differences in the parent mice from which the strains originated, which, in spite of the selective breeding used to establish the pure inbred strains, may persist in extremely diluted traces in individuals.

The results of these investigations show that the strain influence predominated in these tumor cells to such an extent that the regressing nature and the cell individuality were completely masked as long as the tumors were transplanted into mice of the strain in which the tumor originated. When, however, the tumors were transplanted into mice of other strains, the strain influence became submerged and the cell individuality became apparent. Possibly the strain specificity was less paramount and the growth energy greater in these six tumors than in other dibenzanthracene induced tumors, so that grafts of the tumors were able to grow to a moderately large size in mice of other strains before regression took place. Had their strain specificity been somewhat less or their growth energy greater, it is probable that these tumors would have been transplantable in any strain of mice, as was the case with one but only one (C3H-11) of the 294 dibenzanthracene induced tumors (Lewis and Lichtenstein, 1936).

SUMMARY

1. Six of the 294 dibenzanthracene sarcomata induced in this laboratory were found to have an inherent power to grow when transplanted into mice of pure inbred strains. After reaching a moderately large size the tumors underwent regression except in hosts of the same strain as that in which the tumor had been induced.

2. Transplantation of the tumors that grew in alien strain hosts showed that each of these six tumors (BA-1, BA-178, BA-193, C57-220, C57-241 and C3H-263) possessed its own characteristic growth

energy. This proved to be weakest in C57-220, stronger in BA-1, BA-178 and BA-193, and strongest in C57-241 and C3H-263. These differences were masked when the tumors were transplanted into mice of their own strain of origin, since they all grew at about the same rate in every one of such hosts.

3. Striking differences in the growth energy of the tumors were disclosed when their serial transplantation in alien strain hosts were attempted.

4. The growth energy of the tumors was influenced by conditions within the tumor at the time it was transplanted. Grafts of tumors that had begun to regress failed to grow in every one of the alien mice, but all such grafts grew in hosts of the strain in which that tumor originated. On the other hand, grafts of tumors that had almost entirely regressed at the time of transplantation failed to grow even in mice of their own strain of origin.

5. The individuality of the cells of these six tumors was shown by the resistance developed in mice in which a tumor had previously grown and regressed. It was found that mice in which a graft of tumor had grown and regressed were resistant to the growth of another graft of that tumor but not necessarily to the growth of a graft of another of the six tumors, even when the two tumors had originated in mice of the same strain. In other words, resistance was developed against the growth of individual tumor cells, not against the growth of cells of a given strain.

6. Every one of the six tumors retained its strain specificity and grew when transplanted into mice of its own strain of origin, regardless of whether one or more tumors originating in other strains had previously grown and regressed in the implanted hosts.

CONCLUSION

The existence of biological differences in six spindle-celled sarcomata, which from pathological studies appeared alike, was disclosed by the respective growth energy of each when transplanted through one or more generations in mice of the pure inbred A, BA, C3H and C57 strains.

The characteristic inherent growing power possessed by each of these tumors depended in a measure upon intrinsic factors present in the tumor itself, such as strain of the tumor cells and whether the tumor was growing progressively or had begun to regress at the time it was

transplanted; also, to some extent upon extrinsic factors, such as the strain of the host and state of resistance established in the host by the growth and regression of a previous tumor graft.

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THE EFFECT OF FERTILIZATION ON THE RATE OF STAINING OF CUMINGIA EGGS

By

D. M. WHITAKER

From the Marine Biological Laboratory, Woods Hole, and the Department of Biology, Stanford University

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INTRODUCTION

It was found some years ago by Lyon and Shackell (11) and by Runnstrom (13) that after fertilization sea urchin eggs stain more rapidly in certain dyes, but not in others. R. S. Lillie then demonstrated in 1916-18 (5, 6, 7) that sea urchin eggs take up and lose water more rapidly in hypotonic and hypertonic sea water, respectively, if they are fertilized. McCutcheon and Lucke (12), Stewart and Jacobs (15), Whitaker (20) and others have more recently confirmed this, and Stewart and Jacobs (14,15) have shown further that ethylene glycol and a number of related solutes enter the fertilized egg more readily. All of these authors have concluded that the permeability of the egg surface to these substances increases at fertilization, and this is the generally accepted interpretation, although objections have been raised in some instances by Heilbrunn (2, 3, 4). R. S. Lillie (5, 6, 7) proposed that increased permeability at fertilization may be an important factor in the increased developmental activity which follows. The permeability of cells in general often increases with increased activity.

It was proposed by J. Loeb in 1906 (8) and first demonstrated in the sea urchin by Warburg (16) in 1908 that the rate of oxygen consumption increases sharply at fertilization. Numerous investigators have since confirmed this phenomenon in many species of sea urchins [see Whitaker (19)]. Loeb (8, 9) proposed that the essential feature, or one of the essential features, of fertilization and the initiation of development is an increase in rate of oxidation.

Both respiration and permeability to a number of substances increase at fertilization in the sea urchin egg. In the starfish, however, Loeb and Wasteneys (10) found no increase in respiration. This was not regarded as invalidating Loeb's oxidation hypothesis, however, because the starfish egg is a somewhat special case in that the unfertilized egg in sea water is not developmentally at rest since maturation progresses over a protracted period. There is also no increase in permeability to

water in the starfish egg at fertilization [Lillie (7)], and Stewart and Jacobs (14,15) found no increase in permeability to ethylene glycol. The latter authors found indications that permeability increases as maturation progresses.

Although respiratory increase at fertilization (resumption of development) is quite common among different species, it is by no means universal, and Loeb's hypothesis therefore has not turned out to be valid as a generalization [see 19]. The rate of oxygen consumption actually decreases at fertilization to about half in *Chaetopterus* (18, 1) and *Cumingia* (17). Both of these eggs are immature when they leave the female, but in sea water maturation quickly proceeds to the metaphase of the first polar spindle within less than 15 minutes and thereafter the eggs remain developmentally at rest until fertilized.

Since respiration decreases at fertilization in *Chaetopterus* and *Cumingia*, the effect of fertilization on the permeability and staining rate of these eggs is of some interest as bearing on the correlations outlined. Heilbrunn (2) found that the *Cumingia* egg changes volume more rapidly in hypotonic and hypertonic sea water if it is fertilized. He also found that the total amount of swelling or shrinking is greater at equilibrium in the fertilized egg. This difference in equilibrium volume cannot be accounted for on the basis of different permeability, as Heilbrunn points out. He attributes it to reduction in rigidity or mechanical strength of the membrane at fertilization. Heilbrunn goes further and concludes that there is no increase in permeability to water, attributing both the more rapid rate of water transfer and the different equilibrium to decreased membrane rigidity. It appears to me that a more conservative conclusion would be merely that, in view of the different equilibrium, increased permeability to water is not proved. Both *Cumingia* and *Chaetopterus* eggs have relatively tough, tight-fitting membranes that swell at fertilization. These tough membranes complicate the interpretation of swelling experiments.

The penetration of dyes into the egg is presumably not opposed by mechanical rigidity of the membrane as is the entrance of water. Heilbrunn (2) compared the rate of staining of fertilized and unfertilized *Cumingia* eggs in sea water solutions of neutral red and methylene blue. He observed no difference in neutral red, which also shows no difference in the sea urchin (13, 11), and he concludes that there was no difference in methylene blue, although noting a slight

suggestion of more rapid staining of fertilized eggs in the strongest concentration of dye used.

In the experiments now to be reported, the rate of staining of fertilized and unfertilized eggs of *Chaetopterus* and *Cumingia* were compared in sea water solutions of gentian violet, methyl violet, and methylene blue. These same dyes had earlier been found to stain developing fertilized or immature sea urchin eggs much more rapidly than resting unfertilized eggs (Whitaker 20).

METHOD AND RESULTS

The experiments on *Chaetopterus* can be described very briefly. These eggs are very granular and dark and do not transmit much light. In consequence, the intensity of the dyes could not be observed well enough to permit any conclusion, although there was some suggestion that the fertilized eggs stained more rapidly.

Cumingia eggs are much more transparent. The eggs of some females are almost colorless, while those of others are slightly pink. Colorless eggs were used primarily, although the natural color of the pink eggs did not interfere. Each egg is incased in jelly which is invisible in pure sea water. The jelly stains sooner than the cytoplasm, and at least in gentian violet it becomes more pinkish in color than the stained cytoplasm. The jelly was usually removed by pipetting the eggs a few times, although when present, in spite of its different color reaction, it did not obscure the results as the cytoplasm soon stained much more strongly than the jelly.

Twenty-three sets of experiments on eggs from 8 females were carried out between August 4th and 16th, 1938, at the Marine Biological Laboratory. Eggs were kept in syracuse dishes in the laboratory, the air temperature of which ranged from 27° to 29°C. The dyes used were gentian violet (Grüblers), gentian violet (National Aniline and Chemical Co.), methyl violet (Grüblers), and methylene blue (National Aniline and Chemical Co.). Experimental solutions were prepared by pouring saturated sea water solutions through fine filter paper into flasks of sea water until enough particle-free solution had been added to give a lightly but definitely tinged solution, as judged by eye. Solutions of this strength were labelled A. Solutions 3 times as strong were labelled B, and 9 times as strong, C.

After being shed, eggs were left in sea water for from 21 minutes

to 3 hours before being used. This did not affect the results. In order to avoid any interference with fertilization, eggs were not placed in dye solution until 5-10 minutes after insemination, and in 2 experiments the interval was 27 minutes, which did not affect the sign of the results.

Equal concentrations of fertilized and unfertilized eggs from the same sample were dropped into identical syracuse dishes of dye solution almost simultaneously. After the first experiment, which revealed that the fertilized eggs stained more rapidly, the unfertilized eggs were dropped in first to favor them with the advantage, if any, of about 20 seconds. The fertilized and unfertilized eggs were then repeatedly observed under the low power of the microscope. It was found that intensity of color could be judged best when the diaphragm was widely opened and the eggs were flooded with natural sky light even to such an extent that definition was impaired. As soon as it appeared that one class of eggs was more heavily stained, samples of both classes were mixed in a third dish of sea water for direct comparison which invariably revealed even stronger contrast than had been obvious from alternate inspection.

Twelve such tests in A, B and C strengths gentian violet all showed that fertilized eggs stained markedly more rapidly. The difference was clearly detectable in 7-30 minutes in dye, the time being less the stronger the solution.

Four tests in B and C strengths methyl violet gave the same results in 11-28 minutes. One test in A strength gave the same results, while another in A and one in half A strength did not show a convincing difference.

One test in A and two in B strengths methylene blue did not reveal any difference, while one test did after 18 minutes in a solution considerably stronger than C. The contrast was considerably less than in even weak solutions of the other two dyes. This is in agreement with Heilbrunn's (2) results with methylene blue, as he used relatively non-toxic concentrations.

Gentian violet shows the effect most strongly, and in weakest color solution as judged by eye. Methyl violet does not show it quite as well and requires somewhat more strongly colored solutions. Methylene blue does not show it well in any case, and at all only in a solution so strong as to be quickly toxic and damaging.

The dyes are undoubtedly toxic, at least to some extent, whenever present in the protoplasm in visible concentration. Similar degrees of staining in these same dyes did not, however, prevent cell division and early development in sea urchin eggs (20). In *Cumingia*, however, all concentrations which stained eggs heavily enough before the time of the first cell division so that a difference in staining rate could be detected inhibited or largely inhibited cell division. It was necessary to complete the determination before the first cell division because the smaller diameter of the blastomeres reduces the apparent intensity of the stain. Controls cleaved in 50 minutes or less. Cell division in *Cumingia* is especially subject to inhibition by these dyes. Abortive attempts at cleavage were made, and sometimes temporary furrows would pass partly through. In a few cases normal divisions were completed. The protoplasm of the more lightly stained fertilized eggs which failed to cleave, like that of their unfertilized mates, retained a normal appearance and texture for some time, and in some cases the uncleaved fertilized eggs became ciliated and were swimming the next day. In the stronger solutions cytolysis set in some time after the protoplasm had become heavily stained. Often large transparent blisters formed at the periphery. In these cases the fertilized eggs, which had stained more rapidly, also cytolized much sooner.

DISCUSSION AND CONCLUSIONS

Fertilized eggs of *Cumingia* stain markedly more rapidly than unfertilized eggs in methyl violet and especially in gentian violet. These dyes are somewhat toxic so that development is interfered with although not completely prevented. In stronger concentrations, fertilized eggs cytolize sooner. The simplest and most probable interpretation is that permeability to these dyes increases at fertilization. It is, of course, possible instead that the fertilized eggs stain more rapidly and are more quickly damaged because of changed reaction of the protoplasm. In any event, the staining rate in these two dyes, like the protoplasmic viscosity (2), increases at fertilization in *Cumingia* as it does in the sea urchin, although the respiratory rate changes in the opposite direction in the two forms.

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GLUTATHIONE VALUES OF LIVERS AND MUSCLES OF RABBITS AFTER INJECTION OF HYPOPHYSEAL GROWTH HORMONE

By

P. W. GREGORY and HAROLD GOSS

College of Agriculture, University of California, Davis, California

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Gregory and Goss ('34) presented evidence in the rat that when growth is stimulated by daily injections of 1 cc. of hypophyseal growth hormone over a period of several days, the amount of glutathione in the muscle tissue is significantly increased, along with the weight. The hypophyseal growth hormone also influences the glutathione value in liver tissue, though the response is rather variable if the injections are continued several days. According to Goss and Gregory ('35) when rats are sacrificed and analyzed 8, 12, or 24 hours after a single injection of hypophyseal growth hormone, the glutathione values of the livers of those receiving the potent hormone are significantly lower, and this response is marked and consistent. The glutathione values of the muscle tissue were also slightly lower, though this response was less marked. The effects of these single injections were interpreted on the basis of the results of Schaffer and Lee ('35), who found that a single injection of hypophyseal growth hormone decreased the non-protein nitrogen and the peptide amino nitrogen in the tissues of the rat. Schaffer and Lee therefore concluded that this hormone specifically stimulates protein anabolism and that the nitrogenous substances were synthesized into protein. Glutathione is included in the peptide amino nitrogen.

Being interested in analyzing hereditary size and growth in the rabbit we investigated the glutathione response in livers and muscles 12 hours after injection with the hypophyseal growth hormone. The rabbits used were Polish, three-fourths Polish, New Zealand Reds, and hybrids of various sorts. Their ages (4 months to aged) and weights varied widely. Both sexes were employed. The rabbits were carefully paired, however, taking into account breed, weight, age, and sex. In one pair of young hybrids that were litter-mates, a male was paired with a female.

After being paired, the rabbits were fasted 24 hours. One member of the pair was then subcutaneously injected with potent hypophyseal

growth hormone solution. The other member, used as a control, was injected with an equal amount of the same solution in which the hormone was inactivated by heat (Evans, et al '33).

Rabbits weighing less than 1800 grams were injected with 5 cc. of the material; rabbits weighing more than 1800 grams, with 10 cc. Since weight was considered in pairing, each member of an individual pair received the same amount of the injected substance. After injection the rabbits were held 12 hours longer without food. Throughout the fasting period of 36 hours, the animals had access to water. Twelve hours after injection the members of a pair were killed. Samples of liver and muscle, weighing about 30 grams, were analyzed for glutathione and ascorbic acid. The muscle sample was obtained at a definite place from a posterior leg.

TABLE 1

SUMMARY OF GLUTATHIONE AND ASCORBIC ACID VALUES OBTAINED FROM LIVER AND MUSCLE OF RABBITS THAT WERE PAIRED. ONE MEMBER OF THE PAIR WAS INJECTED WITH POTENT HYPOPHYSEAL GROWTH HORMONE, AND THE OTHER MEMBER OF THE PAIR WAS INJECTED WITH A HEAT-INACTIVATED HYPOPHYSEAL PREPARATION.

Breed of Pair	Rabbits Receiving Heat-Inactivated Hormone				Rabbits Receiving Potent Hormone			
	Liver		Muscle		Liver		Muscle	
	GSH mg. %	Ascorbic acid mg. %	GSH mg. %	Ascorbic acid mg. %	GSH mg. %	Ascorbic acid mg. %	GSH mg. %	Ascorbic acid mg. %
Polish	230	0.6	44	0.3	99	9.2	18	Tr
¾ Polish	228	9.7	24	0.3	186	12.3	11	0.3
¾ Polish	154	4.0	23	1.1	187	23.8	10	0.3
Polish	223	3.7	27	Tr	207	11.7	11	0.3
Polish	262	4.3	18	0.9	225	6.0	10	0.6
Hybrid	303	13.5	33	0.3	221	1.7	21	Tr
Hybrid	320	9.4	29	0.3	202	8.3	25	Tr
N. Z. R.	247	3.1	28	0.3	199	3.4	25	Tr
Polish	256	9.4	24	Tr	195	6.9	29	Tr
Polish	204	2.0	23	0.3	232	7.5	18	0.6
N. Z. R.	269	9.7	34	Tr	178	7.5	15	0.3
Polish	227	10.9	25	0.6	189	12.0	13	Tr
Hybrid	195	8.9	37	0.6	123	2.3	37	1.4
N. Z. R.	257	6.6	35	0.3	214	6.0	32	0.3
N. Z. R.	185	9.4	35	0.3	250	8.0	29	0.3
N. Z. R.	230	6.6	31	Tr	223	16.0	25	Tr
Means	236.8	7.0	29.3		195.6	8.9	20.6	

According to results of these analyses, summarized in table 1, the rabbits that received the potent hypophyseal growth hormone have lower glutathione values for both liver and muscle tissue than the controls that received the heat-inactivated hypophyseal preparation.

When livers are considered, the mean glutathione value of the group receiving the potent hormone was 195.6 mg. per cent, whereas the corresponding value of the group receiving the heat-inactivated hormone was 236.8—a difference of 41. The glutathione values of both groups are rather variable. In only three pairs, however, does the

animal receiving the potent hormone exceed in this respect its partner used as a control.

The glutathione values of muscle are also rather variable. The mean concentration of the group receiving the potent hormone is 20.6 mg. per cent, while that of the control group receiving the heat-inactivated hormone is 29.3—a difference of 8.7. Of the individual pairs there is only one in which the glutathione value of the animal receiving the potent hormone exceeds that of its partner receiving the heat-inactivated hormone. In another pair the glutathione concentration of the muscle of each member is the same.

Fisher's method of pairing was used to determine the significance of the glutathione values of the livers and muscles of the two groups of animals. The results of these analyses are summarized in table 2.

TABLE 2

SIGNIFICANCE OF MEAN GLUTATHIONE VALUES FOR LIVER AND MUSCLE OF RABBITS INJECTED WITH POTENT HYPOPHYSEAL GROWTH HORMONE AND WITH A HEAT-INACTIVATED HYPOPHYSEAL PREPARATION. THE DATA ARE OBTAINED FROM TABLE I.

Comparison	t value	t value necessary to be highly significant
Liver glutathione. Potent hypophyseal growth hormone-injected animals vs. heat-inactivated hormone-injected animals.	3.064	2.947
Muscle glutathione. Potent hypophyseal growth hormone-injected animals vs. heat-inactivated hormone-injected animals.	4.552	2.947

It is clearly evident that the animals receiving the potent hormone have significantly lower glutathione values in both liver and muscle than the controls receiving the heat-inactivated hormone.

If the glutathione value of muscle is plotted against the glutathione value of liver for either the group receiving the potent hormone or the group receiving the heat-inactivated hormone, no clear cut relation appears. Any definite relation existing might have been obscured somewhat by the heterogeneity of the rabbits used, which differed widely in age, sex, and genetic constitution for adult size. The individuals constituting a pair were, however, carefully matched before the injections were made.

The ascorbic acid values are relative rather than absolute. In this

report, however, they are calculated as ascorbic acid milligrams per cent--not as glutathione milligrams per cent as in our earlier reports. For liver they are high and rather variable. The differences in value between potent hormone and heat-inactivated hormone groups are slight and insignificant. The ascorbic acid values for muscle of the two groups are low and approximately the same. Several animals of each group showed only a trace of ascorbic acid in the muscle.

As these data clearly show, one subcutaneous injection of potent hypophyseal growth hormone in the rabbit markedly and significantly reduces glutathione in liver and muscle tissue. When the glutathione responses of rabbits and rats to the potent hormone are compared, however, there appear to be specific differences. The rat, according to Goss and Gregory ('35) shows a strong response in the liver and a weak response in the muscle. The rabbit, on the other hand, gives strong responses in both liver and muscle, but the muscle tissue apparently gives the stronger response. To facilitate comparisons between the species and for the different tissues, changes in glutathione values are expressed in per cent of the control. In this way direct comparisons can be made. The animals that received the heat-inactivated hypophyseal preparation are used as controls. These comparisons are summarized in table 3. In the rats receiving the potent hormone the glutathione value for the liver is 44 per cent of that of the control; for the muscle 95 per cent. In the rabbits receiving the potent hormone

TABLE 3

COMPARATIVE CHANGES IN GLUTATHIONE VALUE OF LIVER AND MUSCLE OF RATS AND RABBITS 12 HOURS AFTER INJECTION WITH POTENT HYPOPHYSEAL GROWTH HORMONE DATA FOR THE RATS ARE TAKEN FROM GOSS AND GREGORY ('35).

Species and tissues	Material injected	No. of animals	Mean GSH mg. %	GSH difference	Percentage of control
Rat liver	KGH[1]	15	125.3	69.8	44
Rat liver	PGH[2]	14	55.5		
Rat muscle	KGH[1]	15	33.3	1.8	95
Rat muscle	PGH[2]	14	31.5		
Rabbit liver	KGH[1]	16	236.8	41.2	82.6
Rabbit liver	PGH[2]	16	195.6		
Rabbit muscle	KGH[1]	16	29.3	8.7	70.3
Rabbit muscle	PGH[2]	16	20.6		

[1]KGH refers to the heat-inactivated hypophyseal preparation.
[2]PGH refers to potent hypophyseal growth hormone.

the glutathione value for the liver is 83 per cent of the control; for the muscle 70 per cent. Since, however, the dosage of hormone given rats and rabbits was not exactly the same per unit of weight some of the difference between the species in response to the hormone might be caused partly by unequal amounts of hormone.

The lowering of the glutathione value of liver and muscle in the rabbit 12 hours after a single subcutaneous injection of potent hypophyseal growth hormone may be interpreted on the basis of results obtained by Schaffer and Lee ('35) for the rat. In the absence of critical experimental data for the rabbit it seems logical to assume that the potent hypophyseal growth hormone is stimulating protein anabolism, as in the rat, and that the decrease of glutathione in liver and muscle is negatively correlated with the synthesis of protein. All of our results with potent hypophyseal growth hormone and the results obtained by Schaffer and Lee indicate that there is a definite relationship between the amount of glutathione in the body tissue and increase in weight. Under certain conditions, however, the correlation may appear negative, whereas under other conditions the correlation is definitely positive. If rats fasted 24 hours are then injected with potent growth hormone and the fast continues for 12 hours longer before the analyses are made, the non-protein nitrogen, peptide amino nitrogen, and glutathione (glutathione is also included in the peptide amino nitrogen) decreases in the tissues. Schaffer and Lee ('35) presented evidence that the non-protein nitrogen and peptide amino nitrogen contributed to the new protein that is synthesized.

Under fasting conditions, and with one injection of the potent hypophyseal growth hormone, the amount of glutathione in liver and muscle decreases and the body weight cannot increase even though protein synthesis may be induced from the non-protein nitrogen including the peptide amino nitrogen. Under fasting conditions the correlation of glutathione with growth may then appear negative, since the body weight does not measurably increase. If, on the other hand, rats are injected daily for several days with hypophyseal growth hormone, there is an increase in the food consumption, in body weight, and in concentration of glutathione of the body tissues (Gregory and Goss) ('34). Under these conditions, the amount of glutathione in body tissues is positively correlated with increase in weight. It seems that

the concentration of glutathione may be an index of anabolic activity of the tissue.

Although there are, apparently, certain specific differences in the glutathione response in liver and muscle of fasted rats and rabbits after one injection of the potent hypophyseal growth hormone, both species do respond in the same general manner as regards a consistent lowering of the glutathione in both liver and muscle tissue.

SUMMARY

Thirty-two rabbits were carefully paired as to age, size, breed, and sex. One member of each pair was injected with potent hypophyseal growth hormone, and the other member—used as a control was injected with a heat-inactivated hypophyseal preparation. All rabbits were fasted 24 hours before injection and the fast was continued until they were killed 12 hours after injection. Immediately after killing samples of liver and muscle tissue were analyzed for glutathione and ascorbic acid. The potent hormone caused a significant decrease of glutathione in both liver and muscle. The glutathione response of rabbits and rats to single injections of the hypophyseal growth hormone are compared, and the interpretations of the results are discussed.

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ROOT FORMATION IN SULFURETTED WATERS

By

J. DUFRENOY AND M. VALATX

University of Bordeaux

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Hammett in 1929 postulated that SH is a significant factor in cell proliferation. Correlating the healing properties of the thermal sulfuretted waters at Bareges, as efficient on human wounds, with enhanced cell proliferation, we found growth of root tips to be greater in medium concentrations of those sulfuretted waters than in tap water. However, roots grow in length because cells increase in number, or because they elongate. Hammett and Chapman ('38) found SH specifically associated with cell increase in number as distinct from cell increase in size.

Cuttings from one *Salix viminalis* were rooted: in sulfuretted water from "Tambour" and from "St. Roch" hot springs (Bareges), in water from the cold spring "Puntis", and in tap water.

One experiment (Aug. 3 to 24, 1938) yielded a total of 233 roots on 22 cuttings in the water from the hot springs (Tambour or St. Roch) and a total of 29 roots on 6 cuttings in plain water. The difference of averages $M_1 - M_2 = 10.6 - 4.6 = 6$; t being 1.4, is not significant: P = 0, 20. The difference, however, was of the same order and sign as that obtained the preceding season; namely, an experiment Aug. 16 to 26, 1937 has yielded 2.83 roots per cutting for 12 Salix cuttings rooted in the sulfuretted water from hot springs (Tambour or St. Roch) while 20 cuttings rooted in water from the cold spring Puntis or in tap water averaged 1.35 root, the difference, 2.83 − 1.35, for a t value of 1.7, was also barely significant (P = 0,05).

The significance of the results appears, however, when we compare, not the average number of roots per cutting, but the frequencies of cuttings showing a prescribed number of roots after a prescribed number of days, or better still, the distribution of frequencies of cuttings showing 0, 1 . . . . n roots at the end of the experiment.

An experiment started Aug. 16 gave the following counts on roots per cutting, between Aug. 26 and Sept. 2.

	TAMBOUR											ST. ROCH										
	0	1	3	4	5	6	7	8	9	10		0	1	2	3	4	5	6	7	8	9	10
Aug. 26	1		3	1					1			3	1	1							1	
27	1		1	1	2					1		3		1		1						1
28	1		1	1	1	1				1		3			1	1						1
29	1		1	1	1		1			1		3			1	1						1
30		1	1	1	1		1			1		3				2						1
31		1	1	1		1	1			1		3				1	1					1
Sept. 1		1	1	1		1	1			1		3				1	1					1
2		1	1	1		1	1			1		1	2			1	1					1

	PUNTIS											WATER										
	0	1	2	3	4	5	6	7	8	9	10	0	1	2	3	4	5	6	7	8	9	10
Aug. 26	4		1			1						7		2	1	2	1	1				
27	2	1	2			1						6	1	2		2	1	2				
28	1	2			1	1						3	4		1	1	3		2			
29	1	1	1	1	1	1						2	5			1	3		2	1		
30		1	2			1	1		1				1	3	1	2	2	2	1	1	1	
31			3				1	1	1						4	3	2	1	1	2	1	
Sept. 1			2	1			1	1	1						4	2	3	1	1	2	1	
2			2		1		1			1	1				2	2	3	1	1	3		1

If sulfuretted waters actually enhance cell proliferation, we may expect not only to find more roots per cutting rooted in such waters but the probability of more than one root originating from the same locus on the cutting must be greater than would be the case in plain water.

12 cuttings, numbered T_1 to T_{12}, were rooted in the sulfuretted water

	Number or Roots Originating at same Locus										
	1			2			3			4	
No. of cutting	T	R	W	T	R	W	T	R	W	T	
1	11	11	3	2		1		1	1		
2	18	4	10	2		2			1		
3	10	8	16	3	6			1			
4	12	5	22	3	1	4	1				
5	7	3	12	2	3	1					
6	8	9	4	2	2	1	4				
7	8	11		3			1				
8	8	9		6	4			1			
9	5	7		2	2						
10	11	3		9							
11	15	10		6	3					1	
12	10	8		3	1						
Total	123	88	67	43	22	9	6	3	2	1	

from the Tambour spring; 12 cuttings numbered R_1 to R_{12} were rooted in the St. Roch sulfuretted water, and 6 cuttings (W) were rooted in plain water: the frequencies of 1, 2, 3, 4, roots originating from the same locus are listed above.

Assuming that the frequency of 2 roots issuing from a same locus would be one fourth the frequency of a single root and the frequency of 3 roots would be again one fourth the frequency of 2 roots, the observed distributions for the cuttings rooted in either Tambour or St. Roch waters may be fitted to straight lines (fig. 1).

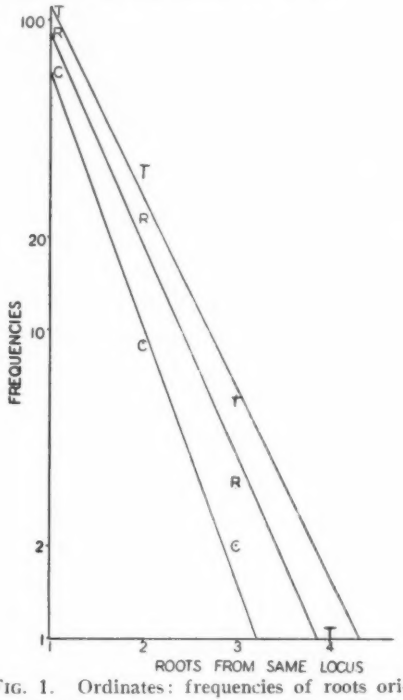

FIG. 1. Ordinates: frequencies of roots originating 1, 2, 3, 4, at the same locus on Salix cuttings; abscissae, number of roots from same locus. C, controls; R, water from St. Roch spring; T, water from Tambour spring.

The frequency of 2 roots issuing from the same locus on cuttings rooted in plain water is only 0,15 of the frequency of a single root, and the straight line adjusted to the observed distribution slopes down more steeply than the two others.

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DIRECT PROOF THAT CANCER AND NORMAL CELLS LIVE AFTER FREEZING AT TEMPERATURES DOWN TO —253° C.

By

J. KLINKE

Biological Laboratory, I. G. Farbenindustrie Aktiengesellschaft Ludwighafen/Rhein, Germany

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Breedis and Furth[1] recently published a communication about the possibility of successful transplantation to healthy mice of neoplastic mouse cells after conservation even for several months at a temperature of —70° C. In addition to sarcoma and carcinoma, the cells of lymphatic and myeloid leucemia could survive for long times in a frozen state. To date several other communications relate the effects of freezing to the transplantability of tumor cells[2]. Koose and Lemmel[3] tried to explant in culture, tissues of mouse carcinoma and Rous sarcoma, after freezing in liquid air for 5 minutes. But they always obtained negative results. The experiments of W. Cramer[4] (1930) were also negative, when he explanted thawed tumor material which had been cooled three times to temperatures of —40° to —20° C. The cells seemed to be killed by freezing. Nevertheless the transplantability of the sarcoma cells was preserved. We have occupied ourselves with the transplantability of mammalian tumors after freezing. Rous sarcoma of fowls, carcinoma of Ehrlich, sarcoma of Ehrlich, ascites-strain of Collier, chondro-sarcoma, melanoma of Passey, Ehrlich-Putnoky-tumors, spontaneous mammary carcinoma of mice, Jensen-sarcoma, Flexner-carcinoma of rats and Brown Pearce-carcinoma of rabbits, conserved their successful transplantability after freezing for various lengths of time in liquid nitrogen at nearly —196° C. It was found by thermocouples, that pieces of tissue about 3 × 2 × 1cm., directly frozen in liquid nitrogen for 2 minutes developed in their interiors the temperature of the liquid gas. The tissues were thawed after freezing rapidly. The rabbit carcinoma of Brown-Pearce showed very distinctly the relation of rapid thawing to successful transplantability. If we thawed at room temperature portions of tumor pulp (30-50 g), frozen for one hour or longer in liquid nitrogen and then made a suspension for intravenous injection in icy Tyrode solution, only rarely did carcinoma develop in the rabbits. But if the same suspension was made by taking

the frozen tissue immediately from the liquid nitrogen into a Tyrode's at room temperature, there always developed a great number of tumors after injecting this suspension intravenously. We could also produce tumors in rabbits by using liquid hydrogen (−253°C) as a freezing agent. In most "round cell sarcoma types" of the mice the influence of the time during which thawing took place seemed less apparent because these mouse sarcoma cells were relatively indifferent to extreme cold. In order to obtain direct proof of survival of tumor cells frozen at extraordinarily low temperatures, above mentioned types of cancer were used in the experiments. I recently published the first results of successful experiments in which liquid nitrogen was used as freezing agent.[6 7]

Technique: Pieces of fresh tissues about the size of rice grains were thrown into liquid nitrogen or in liquid hydrogen. Thawing took place in a few cubic centimeters of icy Tyrode's. For nutritive medium was used fowl plasma plus fowl embryonic extract (diluted 1:2) in the proportion of 1:1. In most cases we explanted very small fragments, less than 1 mm. lengths which were immediately cut from the thawed pieces; they were cultured in a volume of 0,025 cu.cm. of nutritive medium later in 0,05 cu.cm. at 37,8°C). Vital staining and especially emigration and growing out from the explants were used as tests to show survival. The best stains were neutral-red in final concentration of 1:18000 to 1:36000. In comparison to the dense vital staining of fresh control cultures we found mostly only fine, bright red granules, which could be recognized distinctly in the cytoplasm of single cells after incubation of from 1 to 3 hours. Longer cultivation did not usually intensify the vital staining; but generally the neutral-red granules disappeared. The same was seen after longer conservation at room temperature, while by keeping the cultures in the refrigerator the granules were preserved for a long time. Control fragments of tissues heated for half an hour to 60°C. did not show vital staining.

In comparison to the frequency of positive color effects in the tissues which were frozen in liquid nitrogen or liquid hydrogen, positive growth effects were scarce. The most resistant tumor tissue was the above mentioned mouse sarcoma, in which characteristic, spindle shaped cells grew, even if the material had been frozen some days in liquid nitrogen or for $1\frac{3}{4}$ hours in liquid hydrogen. The Jensen sarcoma after freezing and thawing again, also sometimes grew with

charactersitic spindle-shaped sarcoma cells. In cultures of Rous-sarcoma, we obtained but few surviving cells after freezing once in liquid nitrogen, although even this tumor could be frozen about 10 times without losing its transplantability. After freezing growing malignant cell explants, of the well known transplantable carcinomas of the mouse and rat, were even fewer, but the vital staining was nearly always found in single cells. Spontaneous mammary carcinomas of mice, however, were commonly more viable than the transplantable alveolar carcinoma of Ehrlich. Spontaneous mammary carcinoma, directly frozen for 25 hours in liquid nitrogen, showed epithelial growth of characteristic carcinoma cell groups after explantation in several cultures. The fine network of growing stroma cells and numerous cell forms with amoeboid motion were often absent; but sometimes we even saw the formation of a large epithelial membrane. In experiments with liquid hydrogen there were found after freezing for $1\frac{1}{2}$ hours single carcinoma cells with clear vital staining. These results obtained from malignant tissues show that surviving tumor cells of mesenchyme and epithelial type can be found directly after using temperatures down to $-253°C$. But this is not a characteristic of malignant cells, for the same findings were demonstrated with embryonic and differentiated normal cells. After freezing in liquid nitrogen for 5 minutes, thawed fragments of embryonic heart of fowl 7 to 9 days old showed in single cases fibroblastic growth besides vital staining. We saw also emigration of characteristic amoeboid cells, when using frozen embryonic fowl liver. Interstitial cells of cock's and rabbit's testes, especially ovarian epithelium, of fowl embryos and some weeks old fowl still showed vital staining when they had been frozen for 24 hours in liquid nitrogen. In some cases culture of these tissues showed growth of characteristic epithelial cells, in single cases even large membranes. Also clear vital staining after freezing in liquid nitrogen or liquid hydrogen, was shown by the epithelium of kidney as an illustration of its adaptability. The kidneys of 6 weeks old rabbits frozen in liquid nitrogen yielded single cultures in which kidney epithelium grew in large membranes. These results demonstrate that mammalian cells of malignant and embryonic or normal tissues can survive temperatures to $-253°C$.

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THE THEORY OF DIFFERENTIAL PERIODICITY

By

G. F. SLEGGS, M. Sc. (Liverpool)

(*formerly Prof. of Biology, Memorial University College, Newfoundland*)

(*Received June 20, 1939*)

According to the theory of differential periodicity, here enunciated, as far as we know, for the first time, organic form is a projection of the pattern formed by the superimposition of protein lattices. It is already established (evidence summarized by Gulick, 1938; Wrinch, 1936) that a number of proteins have the peculiarity of forming lattice monolayers of great extension; and it is believed that genes may be individual molecules of such types of proteins. It is generally accepted, moreover, that the division of the chromatid is a process whereby a second gene is formed in contiguity to the first. When this process is repeated time after time in the course of growth, the result is equivalent to the building up of a protein lattice. The latter is not formed as a continuous unbroken entity but as a series of isolated fragments. If homologous chromosomes in genealogical succession could be assembled together any given gene would fit with its fellows to form a lattice; and the assemblage of chromosomes would constitute a stack of horizontally arranged superimposed lattices. The lattice layers would not be in actual contact but separated by the subchromatin matrix which alternates between the genes as they are arranged on the chromosome.

We have carried out an examination of the properties of such a stack of lattices, and the result throws light upon certain hitherto obscure properties of the organism. In the first place the system is equipotential. Taking the simplest case possible, namely a 2-layered stack, if the mesh-size, or spatial period, of one layer differs from that of the other, then the stack is differentiated in its radial properties or view by transparency. In certain positions corresponding portions of one lattice will lie vertically in line with the same of the other; in other positions a stagger-pattern will exist (fig. 1). Taking, for example, the vertically projecting amino-acid residues, an upper and lower one, could be directly aligned, so that the matrix could be straddled by a straight line of groupings, but in another position the connection would have to be via a cage work of more complex local construction. The matrix, then, is chemically differentiated to accommodate the amino-

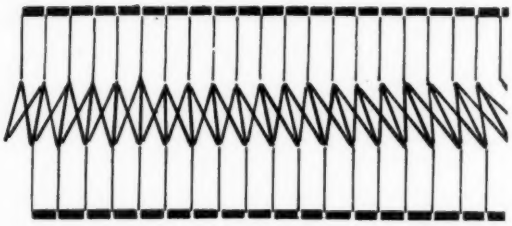

FIG 1. Superimposition of two protein lattices (schematic), amino-acid residues represented by vertical lines. The period or interval is greater in the lower. The synthesizing matrix is represented by joining the ends of the residues, to show that its structure is differentiated.

acid residues which project in great number like the nap of velvet. For the reader unacquainted with biochemical conceptions, differentiation patterns produced in this way can be readily seen by the simple method of superimposing pieces of gauze or plankton netting, when a system of light and dark areas appears.

The periodicity differential can arise in 3 ways: (1) through difference in mesh size; (2) by inclination of mesh (one layer being rotated upon the other) (figs. 2, 3); (3) by bending of the composite layer, whereby layers retaining parallelism now have unequal curvatures.

The differentiation pattern is equipotential from the fact that if a fragment is cut out of the stack (containing at least one mesh of each layer) the whole pattern can be restored if we suppose that it can regenerate new layers. In other words, lateral extension of the fragment restores the whole. The principle of differential periodicity thus leads to the construction of a harmonic equipotential system. Such a system, as is well known, was sought unsuccessfully by Driesch (1907), who concluded that a system with the properties of regeneration exhibited by the organism was mechanistically impossible.

A difficulty not dissimilar in nature has appeared in more recent times in connection with the phenomena of gradient. Child (1915) could see no innate structural basis for the axial gradient, but was in favour of the theory that the gradient was imposed by environmental gradients. Now, if, in a lattice stack, the degree of stagger of the amino-acid residues be plotted in a straight line, a gradient exists. The direction of the line is immaterial, according with Child's further observations (1924) that in an organized cellular field gradients exist

in all possible directions. Moreover, the stagger gradient rises and falls, according to a pattern of repeated waves. If but one wave be present in the field, one has the impression of a gradient running the whole length of the organism; but where the wave length of the differential is shorter, permitting of repeated crests, the gradient may reverse its direction. This fact throws light upon certain of the difficulties admitted by gradientists: thus Spemann (1936) says gradients must presumably be continuous, whereas the occurrence of repeated structures along an axis is discontinous.

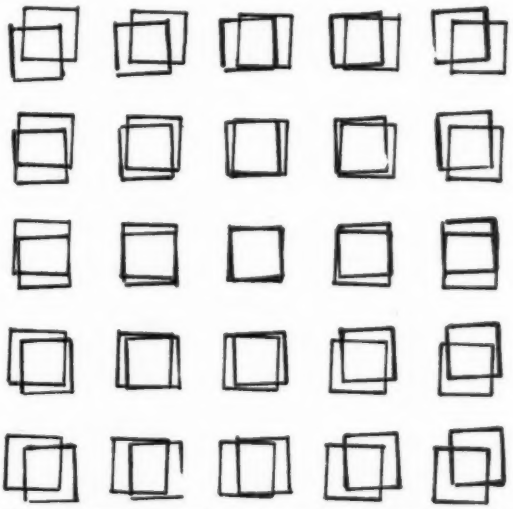

FIG. 2 Superimposition of two identical grids with inclination of mesh. The system is differentiated according to the degree to which units are staggered in different portions of the field. A pair represents a 2-gene cell, the genes in rotational stagger.

The linearity of genar arrangement is the corollary of the tendency to horizontality which arises from the fact that the continual production of new genes from the old represents lattice extension. Further related, is the fact that growth tends to take the form of membranous extension. The form of the organism can only be understood if we consider the genes as assembled to form the lattice stack. A differential equipotential pattern exists between any two adjacent genes, and hence the total pattern, which is an integration of all of these, is highly complex. The

genes may consist of individually different proteins; but it is also possible for the pattern to arise from one protein only if it be the case that the genes are rotationally staggered, (figs. 2, 3).

In any given cell, the genes are arranged in a column, which, owing to secondary causes, is broken up into a number of chromosomes. The column may be intact, however, at the spireme stage. (The complication arising from diploidity is for the moment disregarded). This column represents a shred from the lattice stack. It is differentiated from one cell to another according as to how the amino-acid residues are staggered between adjacent genes, (fig. 4). This results in differentiation of the chromosome matrix occupying the intervals between genes, which determines cytoplasmic differentiation. However, certain degrees of stagger between certain genes will recur in the course of cell division, allowing for the repetition of pigment spots, etc. A cell produces

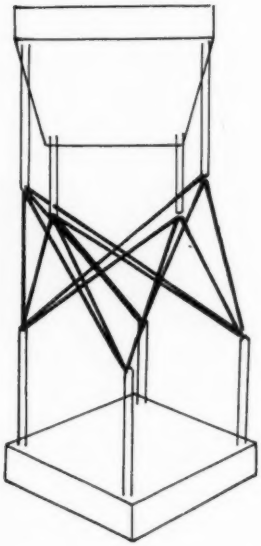

FIG. 3. Two adjacent genes in rotational stagger (schematic). Four residues are shown in each case, the matrix being represented by joining residues. It will vary in kaleidoscope fashion as the genes are rotated.

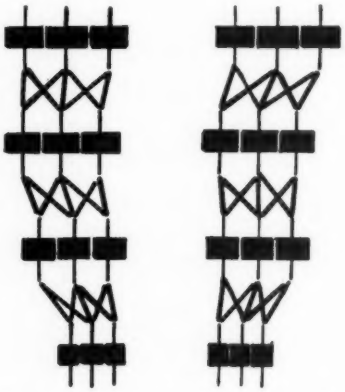

FIG. 4. Division of chromosome. Four genes represented, amino-acid residues as the projecting vertical lines. Matrix represented schematically by joining residues to form a cage work. The daughter genes are equivalent to adjacent portions of protein lattices, so that the residues are staggered in two patterns. The structure of the matrix will differ in order to present the necessary relationship to the residues. The genes are of different mesh and the two chromosomes would fit if brought together.

pigment because two genes have their residues staggered in such a way that the cell is chemically structurized according to a pattern which involves the formation of a pigment substance. Mitosis is a process whereby a nucleus organizes and then repels another differing from itself in the stagger pattern of the genes; this result is produced merely by the formation of new genes in lateral contact with the previous.

Further evidence in support of the theory is derived from a study of the patterns formed when stacks are constructed in the ways indicated on p. 3. Taking two layers, the compound system exhibits conjunction and opposition of properties from one part of the surface to another. This agrees with the development of varying parts of the embryonic membrane into regions of accentuated and reduced growth, and with the fact that highly specialized members of a series are accompanied by reduced or degenerated members, a relationship involved in the law of compensation of parts. A study we have made of organic form suggests that evolution proceeds by the rotation of genes, i. e. by the progressive horizontal inclination of the layers of the lattice stack. We find that the hypothesis of differential periodicity lends itself to the interpretation of organizing action, and of diploidity. It is hoped to present further evidence in succeeding papers.

DISCUSSION

We have been led to advance the foregoing theory as a result of studies upon the problem of equipotentiality. Having ascertained that this property of the organism can be accounted for on the basis of differential periodicity we sought for a mechanism in which the principle could become operative. This mechanism is apparent in the genar system when it is examined by the method of fitting together the genes of the nuclei in a cellular field. The deducible properties of the system, however, have escaped notice from the fact that in actuality the genes do not remain in contact but are widely isolated by the intervening stretches of cytoplasm, etc. The postulation that the genes are flat discs which would fit to form lattices is partly for simplicity in expounding the theory. Even if they could be shown to consist of fibrous or globular proteins, the theory would still hold, providing there are connecting links between the two daughter genes in mitosis to determine accurate spatial relationships at the time of separation. The differentiation of the cell is the result of the changing chemical process which must necessarily be followed in order to synthesize a new gene in struc-

tural contiguity with the old. Were only one gene present, the process could be uniform, the two daughter genes lying upon a uniform synthetic substratum. But if the substratum must synthesize upon its lower face as well as upon its upper, then it cannot be uniform if a periodicity differential exists between the amino-acid or other residues of the upper and lower genes. The genes therefore are arranged in a chain to determine differentiation. The essential is that they be separated by intervening blocks of synthetic cage work. The gene chain or chromatid thus consists of the genes which remain uniform and the matrix which differentiates chemically. The latter draws upon the cytoplasm for various materials in different proportions from one cell to another, and this effect leads to differentiation of the cell as a whole, the nucleus maintaining an appearance of non-differentiation which is partly genuine and partly deceptive.

The total result is that the organism is differentiated into a harmoniouos system of gradients and repeated parts, whose analysis is facilitated by the conception of the various types of pattern which can be formed by superimposing lattices of various forms. If the organs could be precipitated back upon the germ layers from which they arise the same pattern would probably be seen as would be presented could we view the genar proteins in lattice formation.

SUMMARY

A hypothesis is presented which explains the facts of equipotentiality and gradient on the basis of genar arrangement, and behaviour at mitosis. To understand how the genes determine form they must be thought of as brought back from the various cells into lateral contact, as they were at the moments of chromatid division. When so assembled they formed a stack of horizontal superimposed protein lattices. The system is differentiated from the fact that the amino-acid residues are staggered differentially from one part of the system to another. This periodicity differential may result from different distances apart, in the case of different proteins, or by horizontal inclination of mesh, if the genes consist of the same protein. The inter-lattice matrix is therefore chemically differentiated in order to accommodate the residues which enter it from above and below. Hence the intergene matrix is likewise differentiated, determining chemical differentiation in the cell. The properties of the lattice stack vary according to a pattern of property conjunction and property opposition, according with the ac-

centuation and diminution of developmental tendencies from one part of the body to another.

FURTHER WORK. Further applications of the theory—to be dealt with in subsequent papers—may be summarized as follows.

1. *Temperature and Development.* The periodicity differential changes with temperature, explaining fact that form variations occur in organisms which develop over wide temperature range, as plants. In higher animals where form variations would affect viability to greater extent, development must proceed under controlled temperature conditions to ensure fixity of gene differentials.

2. *Control of Body Temperature.* In homoiothermal animal departure from normal temperature alters gene stagger and hence chemical structure of cell, allowing formation of substance (say x) which accelerates or retards metabolism, bringing temperature back to normal, when formation of x ceases, (x occurring probably in limited situation).

3. *Immunity.* Entry of foreign protein distorts chemical framework of cell, restaggering genes so that new framework is formed with which foreign substance can commingle or interact without distortion.

4. *Adaptation and Acquired Characters.* When a cell is altered, as in (3) or in response to other influence, its new shape is now its natural shape—since genes have been restaggered. The protoplasmic cell bridges permit of one cell restaggering its neighbors, the stagger-wave spreading over whole field including the egg. The mechanism thus permits of distortion without tendency to revert as in non-living system. Stated more generally, distortion of the system by environmental change alters the periodicity differential whereby the axis of the organism is reorientated in relation to the axis of the environment.

5. *Non-genetic Characters.* Whereas the differential wave length is determined genetically the position of wave crest may be subject to adventitious influences. Where wave is short, with numerous repeated crests, such variation is unnoticeable. Hence short wave patterns such as butterfly and feather mottlings, show close bilateral symmetry and specific constancy, but where wave is long, producing few coarse blotches as in domestic dogs, cattle and petunia flowers, lack of symmetry and large genetically unconditioned individual variations occur

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THE NATURE OF GEL MEDIUMS AS DETERMINED BY VARIOUS GAS TENSIONS AND ITS IMPORTANCE IN GROWTH OF MICRO-ORGANISMS AND CELLULAR METABOLISM*[1]

By

JOHN W. WILLIAMS

Massachusetts Institute of Technology

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This data is based on observations of Eh and pH in shakes of gel mediums placed in the atmosphere and under varying pressures of gases and their effect on growth of microorganisms and cellular metabolism.

In previous reports (1,2) a gradient of Eh in gel mediums more negative with depth especially in the more alkaline mediums was reported. The practical value of this observation was shown since good growth of anaerobes was obtained when sufficiently negative Eh in lower depths of the medium was attained. Thus, a medium essentially nutrient agar made into shakes except that 0.48 gms. NaOH (better to add 1 gm. dextrose and reduce agar to 7.5 gms./1,000 cc.) was added grew anaerobes well below 12-25 mm. when incubated in the atmosphere; as a rule the growths appeared in one to two days.

The technique of sectioning the shake medium at various depths and the methods for putting the tubes under pressure previously have been described (1,2,3,4). In this work in addition to using bacteriological test tubes, tubes 18 x 400 mm. were used since deeper readings were desired and since in shorter depths of medium especially under increased gas pressures there may be a tendency to equalize Eh and pH throughout the medium. All sterile precautions were taken. Care was taken to introduce electrodes approximately 3 mm. each time since there may be some variation in readings of Eh and pH with different depth

*Contribution No. — from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

[1]See previous MS. (GROWTH. vol. III, no. 1:21): Page 25, line 8 should read: "depth on incubation at 25°C. Page 25, line 27—omit line beginning "The index" and substitute "The index is more comparable to that of growth under 160 lbs. oxygen than under 60 lbs.

of introduction. From 16-20 mm. diameters of medium give comparable results. The mediums were placed at incubator temperature after preparation. Approximately 2 minutes were taken for each reading (tube furnishing 10 secrions would therefore take 20 minutes) and 1 min. readings were taken for interpretations. The Beckman potentiometer which uses the KC1-calomel electrode as reference electrode was used and readings taken in accord with this instrument. Readings were of the same order from almost immediately after solidification for 2 or more weeks; drying of medium and pulling away from the tube apparently finally resulted in their modification. Except where specified the medium consisted of 2 gms. nutrient broth Difco, 1 gm. dextrose, 7.5 gms. agar, 0.48 gms. NaOH/1,000 cc. and it was sectioned and read from below upward; the sections from the tube were put in beakers immediately after the lower end of the tube had been broken off and the medium expelled. Charts which show trends only explain the first part of this work. Jogs in the curves will be noted. This does not mean that even curves were not obtained. They are thus charted purposely since I am as yet unable to explain jogs. Atmospheric gases were not exhausted when organisms were put under pressure.

Chart A. Tubes placed under 200 lbs. oxygen 4 days, read immediately on removal and after $\frac{1}{2}$ and 6 hrs.; 24 hr. readings are little changed from 6 hr. The electrodes are inserted for each reading. The change is at the more negative Ehs. Failure of higher Ehs to reduce to what would be expected (see Chart G) for control mediums in the atmosphere is due to the oxygen retaining capacity of mediums (readings over several weeks or more show retention of this capacity). Boiling and resolidifying shows little effect on this capacity while autoclaving at 15 lbs. for 20 minutes reduces the Eh only to about one-half of what would be expected in tubes placed in the atmosphere with an apparent evolution of gas; autoclaving at 15 lbs. for 120 min. brings medium to normal Eh values. This may explain in part observations of variation of growth of certain organisms when medium is sterilized by filtering from that when medium is sterilized by autoclaving. It may explain in part differences in batches of medium, certain mediums having greater oxygen retaining capacity or retaining more oxygen. Greater oxygen retention may be inhibitory, if sufficient, to many organisms.

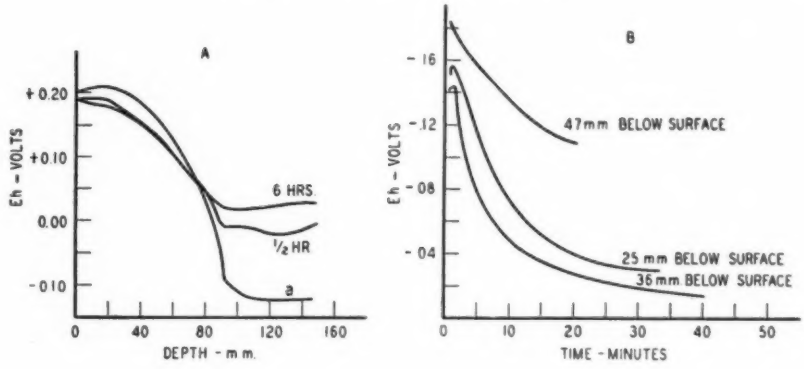
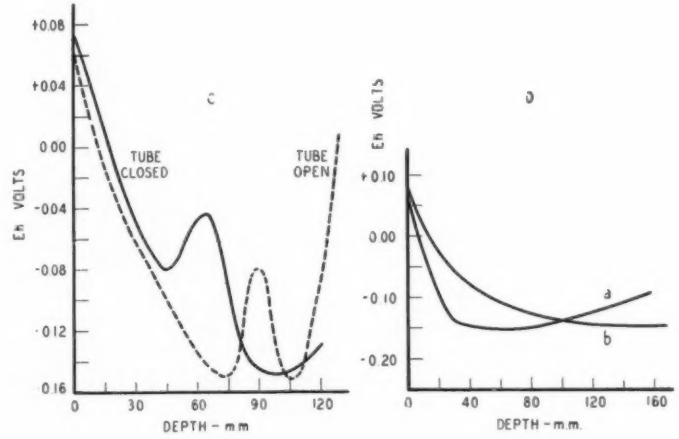


Chart B. Medium stood in atmosphere two days after preparation, sectioned and sites of negative Eh selected to determine speed of Eh increase on standing. Curves show corresponding trend with increase speed at lower Ehs. Decrease of Eh initially as illustrated in 25 and 36 mm. sections has been observed repeatedly. Electrodes were left in situ and not reinserted at each reading.

Chart C. Medium stood in atmosphere two days after preparation before readings. Closed tubes used as control were of usual type. Open tubes instead of being sealed below had cork stoppers. The medium was solified, cork stoppers removed and replaced by cotton plugs. Eh is higher at either end. When tubes were placed under 60 lbs. oxygen,



as would be expected, Eh was higher at either end. This chart shows the importance of the atmosphere with reference to exposed surfaces.

Chart D. Tubes prepared and left in atmosphere two days before reading. Readings begun at top in tube a and at bottom in tube b. Positive surface is not affected but more negative Ehs show increase according to length of time beakers stood in atmosphere. These charts illustrate that significant readings can be made beginning either above or below.

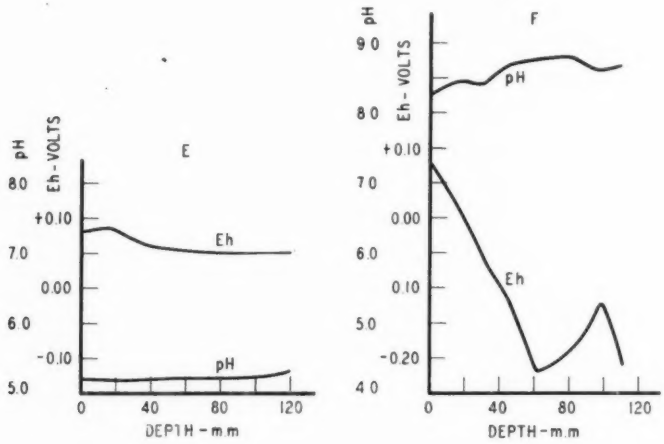


Chart E. Tubes prepared and placed in atmosphere two days before readings. Medium was varied in that instead of adding 0.48 gms. NaOH, 0.0365 gms. of HCl were added. Eh is almost uniformly at high level. As more acid or less alkali is added to medium within limits Eh curve tends to iron out at the higher level. Note pH is uniformly low. Decrease in pH below 5 or above 9 resulted in unsatisfactory solidification.

Chart F. Tubes prepared and placed in atmosphere two days before reading. Simpler medium consisting of 7.5 gms. agar, 0.48 gms. NaOH/1,000 cc. was used. Gradient of Eh as marked as when peptone and dextrose also used is noted indicating the importance of agar which is capable of its production. This may be one reason alkalinized broths are not satisfactory for anaerobic growth. For growth, in addition to agar, nutrient is necessary.

Chart G. Tubes prepared and placed in atmosphere two days before

reading. Variation is not significantly different from F considering a slightly greater alkalinity in F. When 10 gms. dextrose/1,000 cc. is used instead of 1 gm. pH may be several points more acid but Eh is little affected. When dextrose is omitted pH may reach 9.0 and Eh be slightly lower. Eight grams peptone changes Eh and pH little from that of 2 gms./1,000 cc. A slight buffering and poising effect of peptone is probably often present.

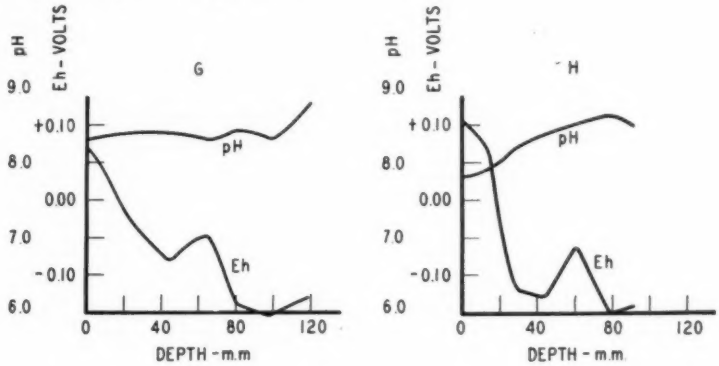
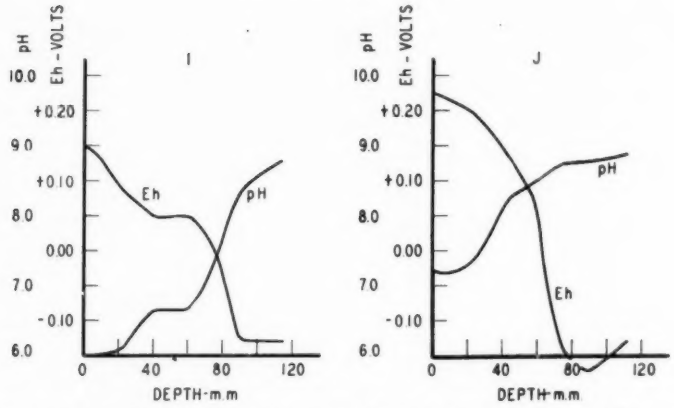


Chart H. Tubes prepared and placed under 60 lbs. nitrogen 4 days. A slight decrease in pH and increase in Eh is noted. Acidity may account for slight variation in Eh. Tubes were also placed under 160 lbs. nitrogen 4 days. A slightly lower Eh and pH were noted with steep early drop in Eh and rise in pH. Variations do not seem sufficiently marked to be considered significant.

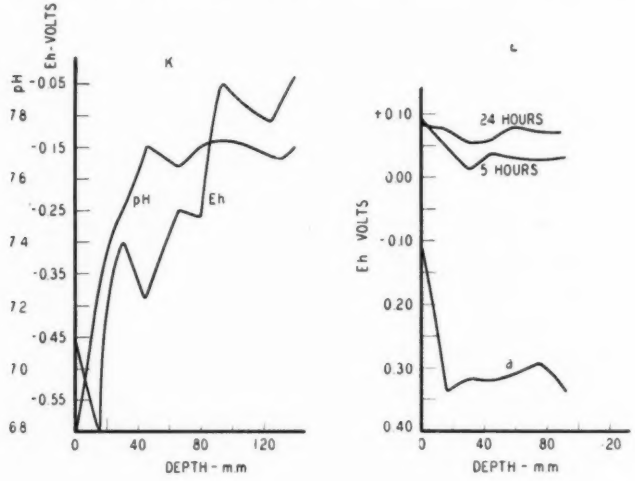
Chart I. Tubes prepared and placed under 60 lbs. carbon dioxide 4 days. Marked variation in Eh and pH are apparent which is more evident when previous charts (E, F, G) are taken into consideration. Acidity change may account for most or all of the Eh change. Tubes were placed under 200 lbs. CO_2 four days. Readings at the surface were about the same as for 60 lbs. but there was a shift of positive Eh about 20 mm. to the right at Eh 0.00 with a general increase in acidity, that near the surface being nearer 5 than 6 with a rise corresponding to the lowering in Eh. The Eh shift to the right from tubes in the atmosphere was 45-50 mm. with 60 lbs. CO_2.

Chart J. Tubes prepared and placed under 60 lbs. oxygen 4 days. A higher initial Eh and lower pH than in atmosphere is apparent. Lowering of pH is not in proportion to that seen for CO_2 but rise in



Eh is in excess. There is a tendency for pH to increase as Eh decreases. The Eh shift to the right is 30 mm. or more greater than that of tubes in the atmosphere, or 15-20 less than with 60 lbs. CO_2 at Eh 0.00. The pH 8.00 with 200 lbs. CO_2 was at 118 mm., with 60 lbs. CO_2 at 85 mm., with 60 lbs. O_2 at 45 mm. and in the atmosphere the pH was above 8.00. When 200 lbs. oxygen was used initial surface reading was no higher but there was a shift in positive Eh about 20 mm. farther to the right; the pH at the surface was similar but that in the depths ranged from 8.0 to 8.1.

Chart K. Tubes prepared and placed under 60 lbs. hydrogen 4 days. The Eh curve follows along the pH curve as though each was influenced

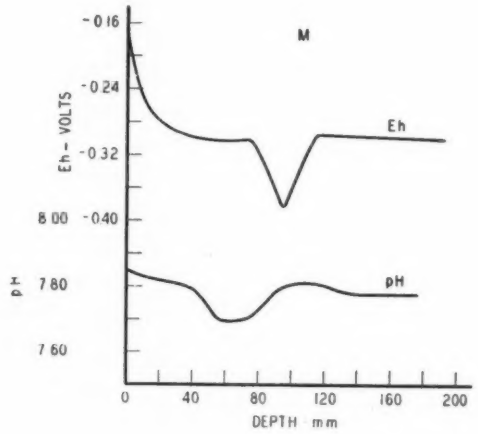


by the same factor. Marked reduction of Eh is apparent. Higher surface Eh value is probably due to greater hydrogen escape after removal from the pressure chamber. Values for lowest depths correspond to what one might expect for like sites in medium placed in the atmosphere.

Chart L. Medium in chart K was examined immediately after removal from hydrogen chamber while that in this chart after standing in tubes 5 hrs. was placed in beakers, examined immediately and after 5 and 24 hrs. Curve a is for beakers examined immediately. Note the negative Eh values indicating equalization. Such equalization has been repeatedly observed under various circumstances. The 5 and 24hr. readings correspond to readings for such mediums in the atmosphere. It is evident that there is no hydrogen reduction fixing capacity on the part of the medium as was noted in the case of oxygen.

Chart M. Tubes prepared and placed in atmosphere 2 days before readings. Medium contained 1 gm. cysteine hydrochloride/1,000 cc. in addition to usual ingredients. The higher Eh at the surface is expected, also a lower general Eh and slightly lower pH. The jog has been noted for many mediums and is discussed elsewhere.

The following series of experiments were performed to show Eh and pH diffusion effects in gels. The mediums used were designated brown and white, the latter containing 0.0365 gms. HC1/1,000 cc. instead of 0.48 gms. NaOH. One medium was solidified in the tube by placing in cold water and the other poured upon it and solidified. There



was a sharp line of demarcation. Examinations were made at intervals after tubes had stood in the atmosphere 1-9 days.

Chart N. White medium above, brown below. Curve for first day is a and 9th day b. Intermediate day readings average between these curves. A slight equalization is observed; this might be expected since the gradient of evaporation is toward the surface and the surface pH trend is toward the alkaline (see Chart P).

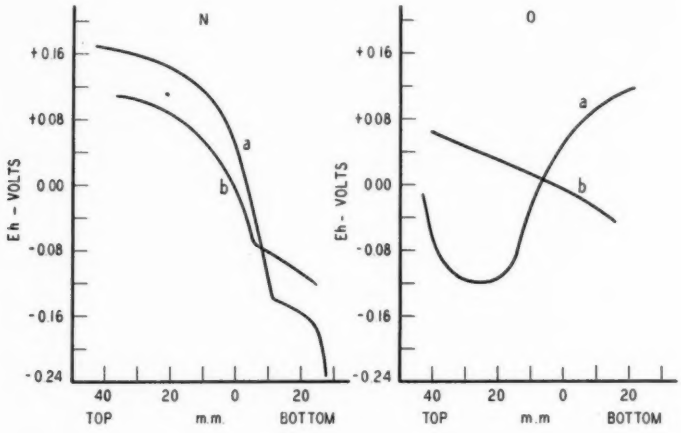


Chart O. Brown medium above, white below. Curve for first day is a and 9th day b. Intermediate day readings average between these curves. There is a marked reversal in the curves, the curve for the 9th day assuming more the position expected for a medium containing a mixture of mediums with both 0.48 gms. NaOH gms. and 0.0365 gms. HCl/1,000 cc. The pH curve (Q) also shows equalization.

Chart P. The pH curve for N.

Chart Q. The pH chart for O.

Curves N, O, P, Q show equalization but that of O and Q is much more pronounced. In N and P Eh change may be dependent on pH, the gradient of evaporation possibly aiding in making this possible. Significant is the apparent protection of the positive Eh area above (with coincident more acid pH) of the more negative Eh below. This in the usual set-up where there are no changes recordable up to two weeks after preparation, where tubes are not influenced by a purposely more acid medium placed below. Change in the upper more alkaline

medium (O, Q) is marked. Gradient of evaporation might explain pH equalization but the marked change in Eh is hard to explain by this alone; absorption of oxygen appears another factor, the more negative Eh possessing an oxygen avidity.

One might assume in N and O a type of respiratory mechanism (here irreversible). If upper and lower ends of curves b are connected their intersection on a is at Eh 0.00 and if upper and lower ends of b in P and Q are connected the intersection is apparently in the vicinity of pH 7.2. These seem the axes about which the changes evolve in the mediums used. These are also probably axial points in many biological reactions.

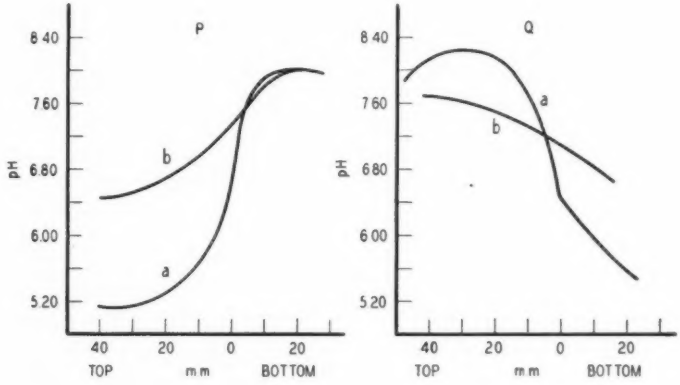


Chart S. A growth spectrum (3, 4, 5) of E. coli communis grown in shake culture under 60 lbs. oxygen for 2 days. Medium consists of 2 gms. nutrient broth Difco, 7.5 gms. agar, 0.04 gms. NaOH/1,000 cc. The variations in Eh and pH are evident. They are greater than control below growth and involve lowering of Eh and pH. Readings above the spectrum are more normal. The importance of hydrogen in growth might be suggested since there is a lowering of both Eh and pH, under increased hydrogen pressure.

As stated previously, application of these studies can be made to growth of anaerobes in the atmosphere, the anaerobes requiring a negative Eh and nutrient for their growth.

Table 1. This gives on the first line after the organism relative upper limit of growth in millimeters and on second line the relative amount of growth. Amount of growth is relative and indicates that

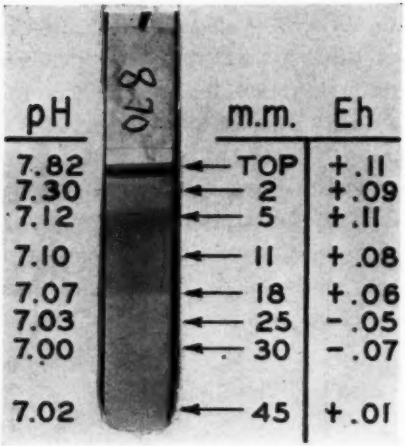


CHART S

growth is good in most instances. The mediums used were 2 and 8 gms. Nutrient broth, 1 gm. dextrose, 7.5 gms. agar, 0.48 gms NaOH/1,000 cc. Growths were often somewhat greater when 8 instead of 2 gms. nutrient broth was used. With Cl. botulinum growth was often better when dextrose was omitted. Gas production was small to absent with 2 gms. nutrient broth but good with 8 gms. In the atmosphere and under 60 lbs. hydrogen growth began at about the same depth, possibly the oxygen above (from atmosphere in chamber when hydrogen was introduced) inhibiting growth in each instance. The greater depth at which growth occurred under oxygen and carbon dioxide is

TABLE 1

	Atmosphere	60 lbs. O_2	60 lbs. N_2	60 lbs. CO_2	60 lbs. H_2
Cl. tetani	15	36	12	31	14
	3+	2+	3+	3+	3+
Cl. welchii	16	36	31	22	14
	3+	2+	3+	3+	4+
Cl. botulinum	14	36*	12	36	14
	3+	2+		2+	3+
Cl. multifermentans	15	37	12	22	13
	3+	3+	3+	3+	4+
Cl. histolyticum	14	76	12	21	10
	3+	2+	3+	3+	4+
Cl. sporogenes	15	35	12	23	16
	3+	2+		3+	3+

*This organism frequently has been noted to grow better without dextrose.

expected since both shift the Eh curve to the right. The growths under 60 lbs. nitrogen do not differ markedly from those under hydrogen or in the atmosphere.

Effects of gas pressures with exception of hydrogen on growth of aerobes have been previously discussed (3, 4, 5, 6). Organisms (in addition to pathogenic fungi) used were: E. coli, B. subtilis, Staph. aureus, S. marcescens, Ps. fluorescens, Ps. aeruginosa, Aerobacter aerogenes, B. megatherium, Proteus vulgaris, B. niger, B. mycoides, Staph. albus, Proteus X-19, B. anthracis (non-virulent), S. enteritidis. Shake mediums used contained 2 gms. nutrient broth, 7.5 gms. agar, 0.04 gms. NaOH. Previously, a proportionately deeper growth on increase in oxygen pressure was reported; with incubation at room temperature growth was slightly deeper and more misty possibly due to more ready permeation of oxygen of colder medium. The misty appearance could be reduced by introducing tubes into pressure chamber cold bringing up pressure and placing chamber in the incubator. Carbon dioxide lb. for lb. allowed deeper but less profuse growth than oxygen. In all instances gases present in atmosphere were present in chamber, gas pressure being added to this. It was reported that under 60 lbs. nitrogen a growth no deeper than 3 mm. and similar to that in atmosphere occurred.

On incubation of the above aerobes in shakes under 60 lbs. hydrogen growths similar to those observed under nitrogen and in the atmosphere occurred.

Mediums buffered at pH 5.5 and 8.0 and mediums similar to that used for aerobes were affected similarly to the more alkaline routine medium of these experiments by hydrogen pressure. The low Eh of surface readings over a depth where aerobic growth occurred indicates that a very low Eh does not prevent aerobic growth as long as the tension of oxygen in the atmosphere is present. Slants of nutrient agar and nutrient broth showed growth of the aerobes as good and with as much pigment as one would obtain in the atmosphere. The Eh of broth under hydrogen was reduced to an extent comparable with the agar shakes. It is not meant to imply that our readings of Eh are as low as those obtained in the chamber. In freshly cut sections an Eh as low as -1.2 has been recorded and in several instances the Eh was off the scale.

Data presented suggests, that since upper limit of growth of anaer-

obes was similar under increased hydrogen pressure to that in the atmosphere, that hydrogen might enter so little into the molecular configurations as not to affect the growth but only a physical state, the Eh. When one realizes that in order to cause hydrogen to react chemically a considerable force, probably greater than that represented in organismal metabolism (or possibly a marked increase of pH) is needed it can be seen why hydrogen might be inert. It seems, therefore, that Eh may measure a state divorced from chemical reactions of metabolism; nevertheless, either this state must reach a certain low range for metabolic reactions to occur or one in measuring Eh is measuring two things—a general state of large aggregates not taking part in intimate organismal metabolism and a state of the molecules reflected in the general state. In comparison, an object may be hot as a result of the surroundings from which it is taken, or it might be hot as a result of activity of its molecules. Since my mediums do not retain the hydrogen what is measured seems comparable to the former general state and under the conditions the physical state might be without influence.

Another angle from which the problem must be considered is that the platinum electrode may be unsuitable for measuring Eh, possibly because it catalyses hydrogen. If so, it must be shown that substances in the medium responsible for reduced Eh in absence of hydrogen are not catalysed by platinum or that the catalysis is of a different order. Experiments are now being planned using the gold electrode as a substitute.

Oxygen presents a different problem. It is active with no greater forces than those of metabolism and is a part thereof. As certain substances may be anhydrous so apparently may they be anoxic. This avidity is such that apparently forces comparable to increased pressures of certain gasses may be necessary to satisfy it. With too great satisfaction of this avidity there apparently is false denaturation as far as ability of certain microorganisms to grow is concerned. The Eh at upper levels increases not supporting growth under increased pressure but when such medium is melted, planted as shakes and incubated in the atmosphere normal growth occurs. The melting is not a sufficient force to markedly lower Eh toward a level one would find had the medium not been put under oxygen. The slight drop in Eh when oxygen pressure is released with subsequent reading seems to indicate oxygen not "adsorbed"; it is possible to visualize a general and

molecular state in the instance of oxygen as with hydrogen. The general state may prevent growth at upper levels when pressure of oxygen is increased or with certain mediums. The medium-atmosphere relationship with reference to depth of growth has been discussed elsewhere (6). It seems an over-abundance of oxygen may prevent normal reactions of oxidation-reduction necessary to promote growth.

Effects of carbon dioxide have been considered due to acidity. Carbon dioxide is about 30 times as diffusable as oxygen, increases acidity (which increases diffusion of oxygen) and therefore must increase diffusion of oxygen. The more positive Eh at upper levels of medium under increase carbon dioxide tension may therefore be due to an increased diffusion of oxygen; this might be comparable to what occurs with increased oxygen pressure and account for deeper growths of aerobes and anaerobes than in the atmosphere. While conceded, carbon dioxide is inhibitory to aerobes, this feature should be investigated further. It has been demonstrated previously (4) that when growth is established premeability to oxygen apparently increases. Thus with carbon dioxide the normal process of oxidation and reduction may be interfered with.

Changes discussed can be considered due to variation in oxygen "adsorbed" and "unadsorbed" to molecular aggregates of gels. One possible discrepancy, however, presents itself. When aerobes and anaerobes are mixed the anaerobes grow up under the superficial aerobic growth closer to the surface. If growth had produced greater permeation of oxygen this would not be expected. The experiments from which it was concluded that growths probably produced a greater permeability of oxygen were of a different order (4). Aerobes were first grown in the atmosphere in shake culture for 48 hrs., subsequently placed under 60 lbs. oxygen for 48 hrs. with a result that a much deeper growth spectrum was produced than would be obtained if growth had not previously been allowed to occur in the atmosphere. With mixture of aerobes and anaerobes we did not increase oxygen pressure, had a gradient of evaporation toward the surface and a reduction below the aerobe out of proportion to increase in acidity; in other words, the forces acting against permeability of oxygen seem in excess and there is no increase in oxygen pressure making growth of anaerobes farther upward toward the surface impossible.

The ability of broths to establish a gradient of Eh needs further in-

vestigation. A few aerobes have shown rings of growth below the surface similar to precipitin rings when grown under increased oxygen pressure. Whether broth without agar can establish a gradient remains to be shown. Technical difficulties as currents resulting in diffusion present themselves.

CORRELATION

Data presented can be correlated with the living biological mechanism. The skin may be selected. Crudely, the characteristics of the surface of the more alkaline medium may be pH 8.3, oxygen tension 150 mm. and Eh +0.08, of the more acid medium pH 5.2, oxygen tension 150 mm., Eh +0.08. A 20 mm. depth of the more alkaline medium may have pH 8.5, oxygen tension decreased (no satisfactory method of measuring), Eh −0.12 and of an acid medium pH 5.2, oxygen tension possibly little if at all decreased, Eh +0.08. The epidermis at its surface is reported to have a pH 5.6, oxygen tension 150 mm., an Eh possibly +0.08 and in its depth a pH approximately that of blood or 7.4, oxygen tension possibly near that of capillaries of 60 mm. and a negative Eh as indicated by the proliferating Malphigian layers of cells.

In the skin and medium the gradient of diffusion is toward the surface by evaporation. In the skin possibly carbon dioxide is given off to as great an extent as oxygen is absorbed. It is estimated that 1% of respiration occurs by the skin. Estimating the weight of the skin indicates that this 1% of respiration could care for the needs of the skin. Respiration takes place through the surface when aerobes are growing in shakes.

The effect of carbon dioxide in increasing respiration is considered due to the increased acidity. Increase in oxygen tension within limits produces no effect on respiration. Coincident with variation in pH are variations in Eh. Whether they are sufficient to affect bodily mechanisms remains to be demonstrated. While our results are suggestive they are with high pressures and more refined techniques are necessary to draw correlations closer.

Buffered mediums have been used in only a limited number of instances. There has been some difficulty in satisfactory buffering. Added factors are introduced and more study will be necessary.

The value of the more alkaline medium does not seem limited to Clostridia. "Anaerobic" streptococci, mixed cultures from Vincent's showing fusiform organisms of many forms have been grown and

suggest adaptations. Treponema pallidum* carried in rabbit testicles have been planted in the depths of the more alkaline mediums. Possible spiral forms when Difco brain heart infusion broth was substituted for nutrient broth have been observed from growth 2-8 mm. below the surface. Animal experiments are in progress.

SUMMARY

Data is given in charts which illustrates trends and practicability of my method of study of Eh and pH in gels for learning more about gels so that their function and the function of ingredients they contain may be better understood in investigations of living tissues and organisms which grow in and upon them.

Data is given illustrating the effects of gases and tensions of gases on gels and on microorganisms growing on and in them.

Correlations of the above studies with the skin and mechanism of respiration are suggested. Similar correlations can be made with other living systems.

Suggestions as to possibilities in further investigations are presented.

Suggestions as to explanations of variations noted in growths on mediums sterilized and prepared differently are given. The ability of mediums to retain oxygen in contradistinction to their inability to retain hydrogen is discussed.

It must be realized that there are many variables in experiments such as these. I have attempted to give generalities in which the variables seem to have been satisfied as a point of departure for further studies.

A discussion which indicates that all changes noted may be dependent on "adsorbed" and "unadsorbed" oxygen is presented. Possibly too many, although pertinent, assumptions have been made in this discussion

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*I wish to express my thanks to Dr. William A. Hinton, Director of the Massachusetts Wassermann Laboratory, for the assistance he is giving me in this phase of my experiments.

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PROPORTIONAL LENGTH GROWTH OF GAR
(*LEPISOSTEUS PLATYRHINCUS DE KAY*)

By

F. S. HAMMETT and D. W. HAMMETT

Marine Experimental Station of The Lankenau Hospital Research Institute
North Truro, Massachusetts

(*Received for publication July 12, 1939*)

By courtesy of John Bass, Jr. of the Bass Biological Laboratory, Englewood, Florida, we were given a catch of Gar in sufficient size range and number to justify growth study.

Such seemed worth making, not only for intrinsic interest, but also because this fish apparently represents a form which has persisted from the palaeozoic to the present. As such it might give information regarding the relative growth pattern of ancient and more modern species.

The 225 fish obtained from a road-side muddy slough were identified as *Lepisosteus platyrhincus De Kay* by Mr. Springer of the Bass laboratory. They ranged in total length from 39 to 133 cm. The catch comprised 124 stripped fish and 101 spotted.

Although it is opined that spotting and striping indicate age difference* it may be questioned whether a 43 cm. striped fish is really older than a 132 cm. spotted; particularly when the total-length range and distribution in the two populations is essentially the same.

Because of this it seemed the two types might represent two varieties and length records were made thereof separately.

Measurements were made according to the U. S. Bureau of Fisheries methods with meter-stick and calipers. Records were taken of total, head, body, and tail lengths. Standard length was gotten by adding the values for head, body and tail; caudal fin length by subtracting this from total. Measurements were made on live fish only. Thus distortion and shrinkage from rigor or fixative were avoided.

An exhaustive comparison of the two types with respect to length progression in absolute and percentage values failed to reveal any sure difference in relative growth. Because of this the combined data are used for analysis unless otherwise noted.

It would take much space for no useful purpose to print the single

*Personal communication from Professor Carl V. Hubbs.

TABLE 1
LENGTH DIMENSIONS OF 225 LIVE GAR (LEPISOSTEUS PLATYRHINCUS DE KAY) FROM ENGLEWOOD, FLORIDA
ARRANGED BY 10CM. CLASS-RANGE INCREMENTS IN STANDARD LENGTH

	Class Range STANDARD	No.	Total	Standard	Head	Body	Tail	Caud. Fin
1	30 — 39.5	13	43.8	37.6	10.7	20.4	6.4	6.3
2	40 — 49.5	69	52.0	44.8	12.9	24.1	7.8	7.2
3	50 — 59.5	50	62.6	54.1	15.4	29.0	9.7	8.4
4	60 — 69.5	36	74.3	64.5	18.2	34.8	11.6	9.8
5	70 — 79.5	29	84.3	74.1	20.7	40.7	12.7	10.1
6	80 — 89.5	15	95.9	84.0	23.2	46.5	14.4	11.9
7	90 — 99.5	9	105.1	93.1	24.9	51.5	16.7	12.0
8	100 — 109.5	2	121.0	106.0	28.0	59.5	18.5	15.0
9	110 — 119.5	2	132.5	116.0	31.5	64.5	20.0	16.5

measurements. These are on file at the Institute and available to the student. Instead the raw data are presented in Table 1 as class-range averages arranged by 10 cm. increments of Standard Length. Distribution in smaller (5 cm.) increments is cumbersome and gives no additional information. Standard length is recommended as the reference basis because damaged caudal fins may distort Total length significance.

Visualization is found in Chart 1. The same result ensues when data are plotted against total length. It is evident that growth of each part is in essential straight-line relation with that of the whole. The equations are given in Table 2.

The course of growth is the same as that reported for other fish by Crozier and Hecht ('13), Hecht ('16) and others cited thereby. Hubbs ('25) and Hubbs and Whitlock ('29) on the other hand report curvelinear relations, though the conclusion is qualified by the statement that "——the dimensions of the parts of a fish throughout the greater

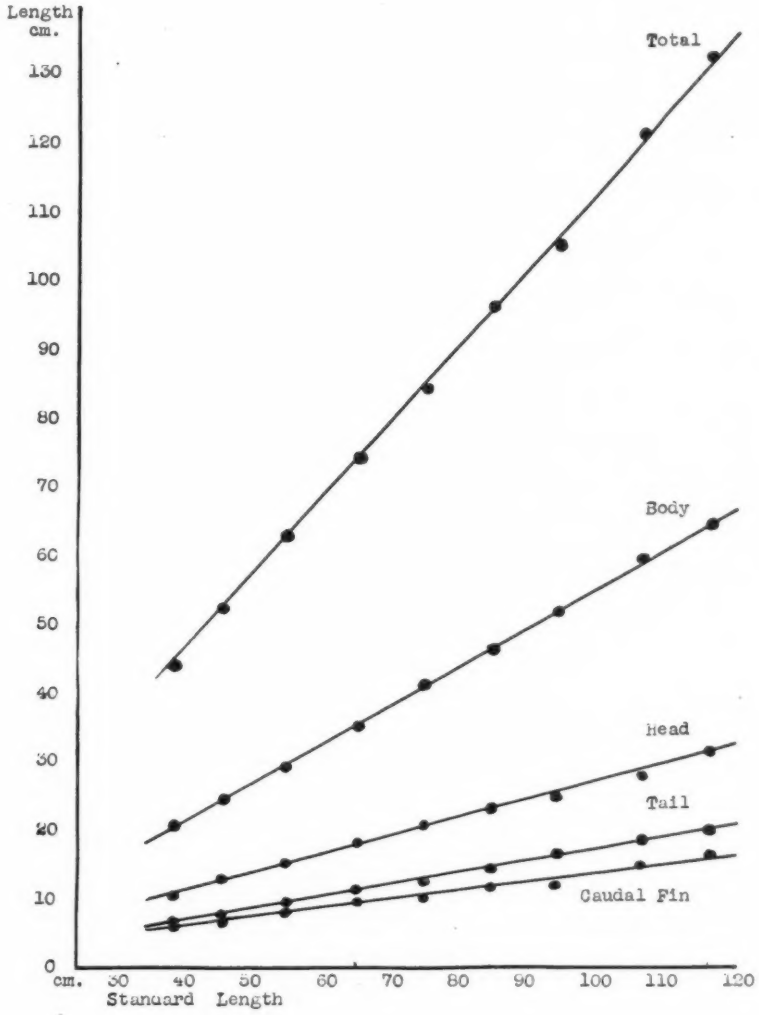
TABLE 2
EQUATIONS FOR LENGTH GROWTH OF 225 LIVE GAR
(*Lepisosteus platyrhincus de Kay*)
ON
STANDARD LENGTH INCREASE

$y = a + bx$

Total	$y = 1.4884 + 1.1245x$
Head	$y = 1.4166 + 0.2562x$
Body	$y = -1.5468 + 0.5709x$
Tail	$y = 0.0956 + 0.1735x$
Caud. Fin	$y = 1.4975 + 0.1242x$

Chart 1

LENGTH ON STANDARD LENGTH
225 Gar (Lepisosteus platyrhincus de Kay)



portion of its life-history do bear a relation closely approximating that of the linear to the total length."

Since Hubbs' 1929 material "had been well preserved, first in

formaldehyde and later in alcohol" one might suspicion this as a factor contributing to curvelinear recording on the assumption that the smaller the fish the younger the fish, and the younger the fish the more tissue water to be lost in fixation.

Despite most meticulous examination of our data we are unable to find any evidence that *Lepisosteus platyrhincus de Kay* undergoes what is designated as a "growth cycle" during its 300 per cent length growth from 38 to 116 cm.

Linear relation of part to the whole is not unique. It is found in fetal growth (Scammon, '20; '21); in cattle (Brody '27); and even in growth of plant parts (Sinnott '36). Despite present enthusiasm all relative growth is not logarithmic.

Head length growth however does not maintain a constant slope relation to Standard length increase. A deviation to a lesser slope occurs. This is shown in Chart 2. It is real since it occurs in both the striped and spotted types separately, and since statistical analysis shows the Difference of the Slope Means is more than three times its Probable Error. Exposition is found in Table 3.

TABLE 3

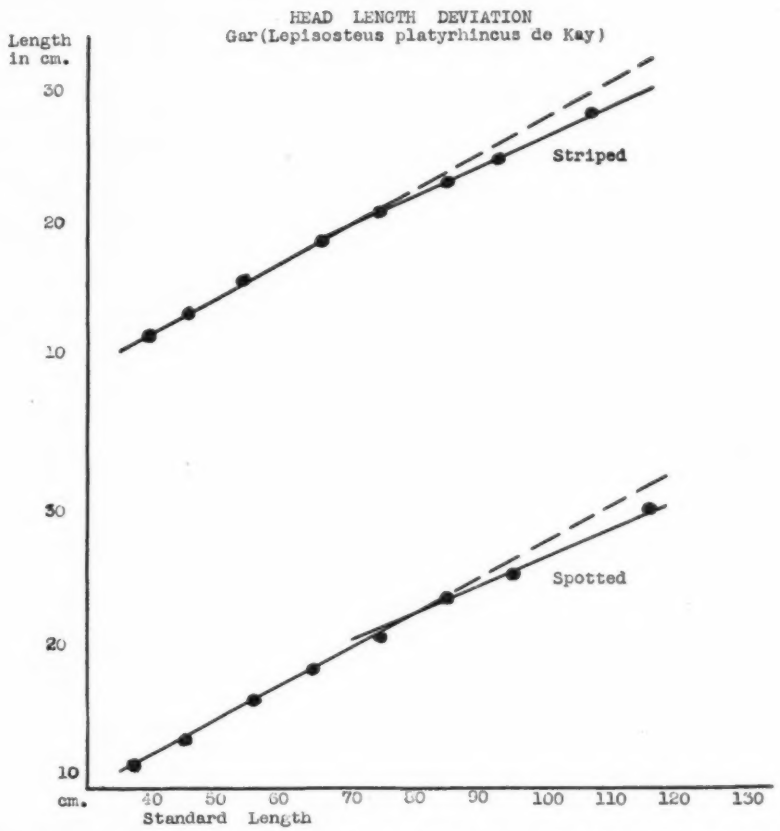
THE EQUATIONS FOR HEAD LENGTH GROWTH ON INCREASING STANDARD LENGTH; THE AVERAGE DEVIATIONS OF THE OBSERVATIONS THEREFROM; THE STATISTICAL ANALYSIS OF THE SLOPES (b) AND THE DIFFERENCE BETWEEN THE PRE- -AND POST-—DEVIATION SLOPES

LIVE GAR (*Lepisosteus platyrhincus de* Kay)

Type	y = a + bx	SLOPE (b)			Comparing A and B		
		Av. Dev.	Stand. Dev.	PEM	Diff. Means A – B	Prob. Error Diff.	dm/ /PE dm
		All Observations					
Striped	y = 1.8795 + 0.2491x	0.21	0.00488	0.00124			
Spotted	y = 1.7247 + 0.2506x	0.33	0.00679	0.00173			
		A. Growth Before Deviation					
Striped	y = 0.7002 + 0.2719x	0.05	0.00168	0.00057	0.04635	0.00081	57
Spotted	y = 0.4647 + 0.2737x	0.11	0.00227	0.00069	0.06246	0.00176	35
		B. Growth After Deviation					
Striped	y = 4.0111 + 0.2256x	0.10	0.00149	0.00058			
Spotted	y = 5.5981 + 0.2112x	0.23	0.00339	0.00162			

Head length growth subsequent to the deviation remains in straight line relation to standard length increase. Thus the deviation is not an example of logarithmic growth. This is sustained by the closeness of adherence of observation to the straight line equation; the low value of the average deviation therefrom; and the fact that this value as well as that of the Standard Deviation of the Slope Mean is reduced from that

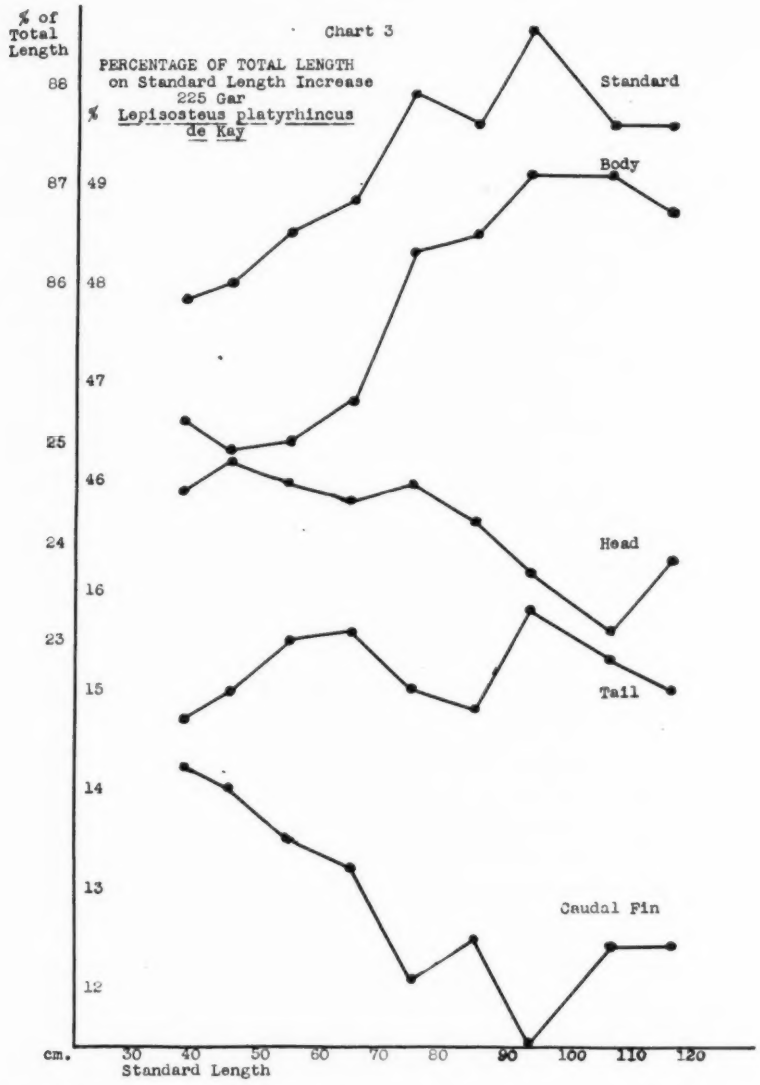
Chart 2



obtaining when head length growth is taken as undeviating (Table 3; Chart 1).

Hecht ('16) expresses the belief that "——the form of the fish is constant——", "——that the rates of growth of all parts of the fish are identical." Hubbs ('25) and Hubbs and Whitlock ('29) intimate that form does change as growth progresses. Our data sustain the later postulate. Chart 3 shows the percentage value of each measured part to Total length as Standard length increases (taken from the basic data of Table 1).

Clearly Standard length, which is made up of Head, Body, and Tail length, becomes a larger and larger proportion of Total length, while



a balancing Caudal Fin becomes a smaller and smaller part.

When Standard length is broken down it is found the greater part of its proportionate increase is due to the Body length component with Tail length running a very very weak second. Compensatory is the decrease in Head length percentage.

This in and of itself is sufficient evidence that the form of *Lepisosteus platyrhincus de Kay* is *not* constant and that the rates of growth of all parts are not identical.

The course and inclination of the percentage progression would suggest the order of decreasing growth rate is; Body, Tail, Head, Caudal Fin. This is sustained by the total percentage increments as well as by the percentage growth capacity of each successive class range which may be computed from Table 1.

The less rapid Head length growth is similar to the finding of Hubbs ('25) with very young *Trachypterus rex-salmonorum.* Tail length preponderance however is not here exhibited as it was with the

Chart 4

HEAD, TAIL, and CAUDAL FIN
Length on Body Length

225 Live Gar(Lepisosteus platyrhincus de Kay)

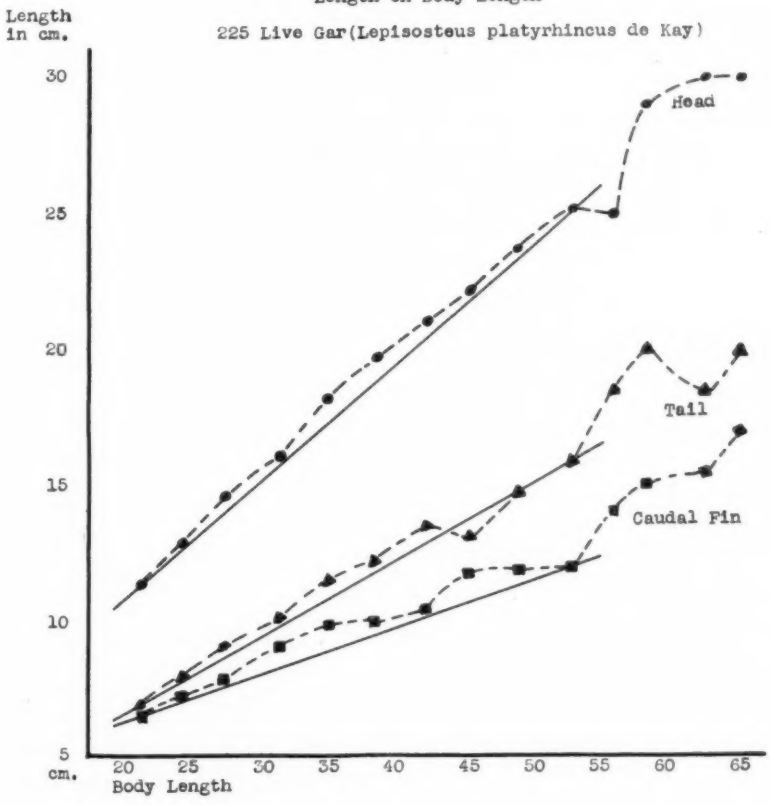
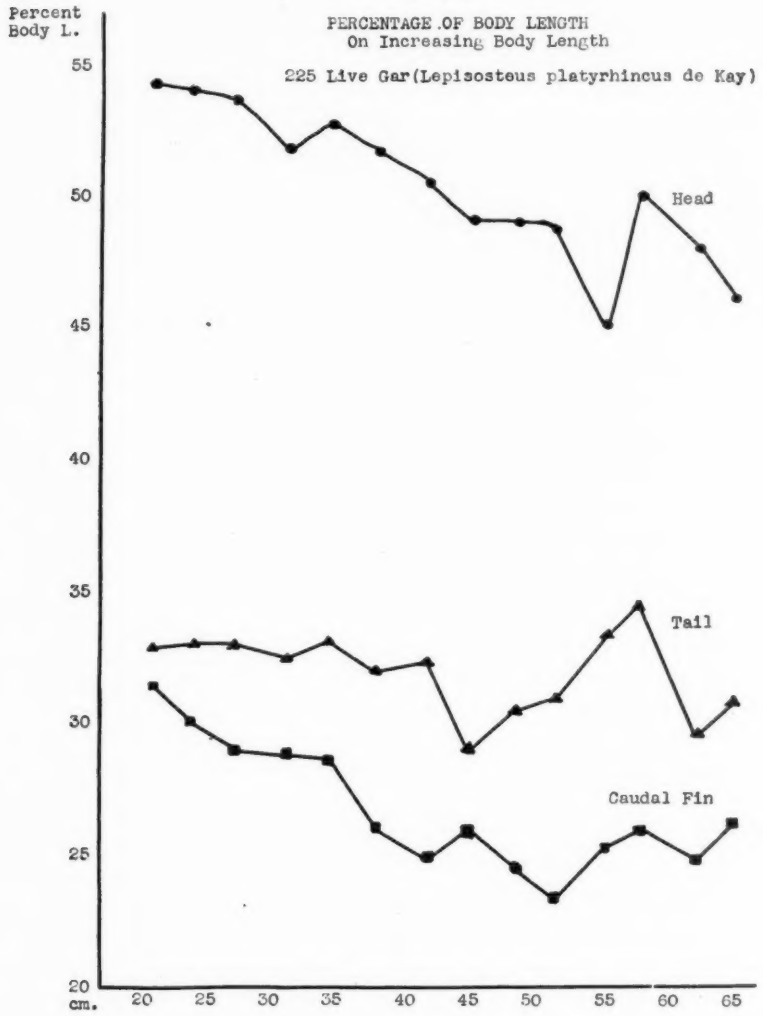


Chart 5



ribbon-fish. Perhaps because our fish were well beyond the larval stage.

So far we have been dealing with relation of part to whole. Such comparison has elements of double jeopardy since the part is in part being compared with itself; values of the whole being inescapably con-

ditioned by those of the part under comparison. It is possible to dispense with these potential mathematical interferences by comparing part with part. We have examined the length growth of head, tail, and caudal fin in terms of that of body. Chart 4 shows that length growth of these parts is not in sure straight line relation to that of the body. A trend to convexity is exhibited in each case throughout the region of adequate numbers; i. e. from 21.0 to 51.5 cm. mean Body length. Above this range the number of fish (5) is too small to allow decision.

Computations of equations to fit these data is hardly worth while. Charts 1 and 4 show all that is sufficient and necessary for the present; viz. that growth of part relative to the whole does not necessarily follow the same type of course as growth of part relative to another part. From which it follows as stated elsewhere that relative or any other growth is hardly expressible by a single formulation (Hammett '36).

As set forth in an earlier paragraph and indicated by Chart 3, tail, head, and caudal fin length growth is less than that of the body in the order named. This decision is sustained by the change which occurs in the tail—, head—, and caudal fin-body length ratios as body length increases. As Chart 5 shows these become less and less, the greatest proportionate decrease taking place in the caudal fin; the least in the tail. Consistent are the total percentage increment values and the percentage growth capacity values for each successive class range.

DISCUSSION

Assuming the samples measured by Hecht and by Hubbs were adequate, and knowing that that of the Hammetts was since it comprised essentially the population of the entire pond which was seined; and assuming that lepisosteus platyrhincus really is a species of great antiquity while those of Hecht and Hubbs were more recent, opportunity for interesting speculation is opened.

In age old lepisosteus platyrhincus there is combined an essential straight line length growth relation of part to whole, with a change in form or length proportions of parts to whole, as total or standard length increases.

Neither of the two groups of fish studied by Hecht and by Hubbs are reported to possess these two growth properties in combination. Those of Hecht grew in straight line relation of part to whole, but did not change in form. Those of Hubbs changed form but the length

growth relation of part to whole tended towards the curvelinear.

It is possible to see in this disjunction the working of that specialization which is the definitive aspect of evolution. There really is no reason why the basic property of growth should not be responsive thereto as are other activities and developmental processes, factors, and participants (Hammett '36) it should be as it undoubtedly is the *sine qua non* of specialization expression.

There is no question but that stripedness and spottedness divided our lepisosteus platyrhincus population into two groups. There is no question that the difference is not a size distinction. It follows that it is not an age distinction unless size in this species is unrelated to age. It may be a sex difference. Unfortunately we have no data on this point. On the other hand it may be a species or variety difference. The fact the two groups show growth concordance would not necessarily rule out this possibility (Hubbs '29). Environmental difference (Hubbs '34) is ruled out since all came from the same slough. It remains for future historians to settle the meaning of stripedness and spottedness. All we can do is to point out and emphasize its existence.

One of the saddest fallecies in the field of growth is the elevation of time to a factorial pedestal (Glaser '38). Time does not produce growth. Growth is produced by incorporation and reconstitution of new substance into that already present. Time does not produce form. Form is produced by cell increase in number, cell increase in size, by cell substance and function. Too often the moving shadow is taken for the substance. Time is but a yardstick for measuring duration of or interval between events. It is not the event itself nor does it determine or even condition the event. The event is predetermined by the substance. Time of and by itself is unimportant. What is important is sequence. The event of the event. The parents of a cretin are rightly more concerned over the breakdown in developmental sequence than over the years.

In these lepisosteus studies time is of no avail because it is unknown. Yet without knowing time an adequate understanding of length growth of this fish has been had. This raises the suspicion that the super-refinements of mathematical treatment of growth data may be more useful as exercises of the intellect than as elucidators of growth processes.

SUMMARY AND CONCLUSION

Length measurements were made of the head, body, tail, and caudal

fin of 225 live Gar sp. *Lepisosteus platyrhincus de Kay* over a total length range from 39 to 133 cm. collected from a single slough in Englewood, Florida.

Two types were present, a striped and a spotted. No sure difference in growth progression obtained between the two.

The length growth of the single parts relative to that of the whole and standard was in straight line relation.

The length growth relation of single parts to that of the body tended to be convexly curvelinear.

Thus no single formula is adequate to express relative growth.

The several parts differed with respect to their growth expression with the result that the form of the fish changed with increase in total and standard length.

The order of decreasing length increment with increase in Total length was: Body, Tail, Head, Caudal Fin.

The possible significance of these recordings is discussed in the text.

Appended is a bibliography of Gar which was prepared through the courtesy of Dr. E. D. Reid of the Smithsonian Institution.

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A PRELIMINARY STUDY OF ELECTRICAL CORRELATES OL GROWTH IN OBELIA GENICULATA

By

H. S. BURR AND F. S. HAMMETT

The Yale University School of Medicine and the Marine Experimental Station of the Laukenau Hospital Research Institute

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The colonial-living form of *Obelia geniculata* has been used for the past eight years as a test animal in studies of naturally occurring chemical factors of developmental growth (Hammett '33 *et seq.*). It is especially suited for such investigation since any good colony of 20 or more individuals has potentialities for 34 separate growth expressions during a 24 hour course of experiment. Thus there are 7 of initiation; 8 of proliferation; 10 of differentiation; and 9 of organization. In addition there are changes incident to regression and senile dissolution (Hammett and Schlumberger '37).

Since chemical agents play a large role in these phenomena and since many chemical reactions are accompanied by electrical changes, it is important to learn whether or not electrical correlates are to be found in this animal as they have been in the salamander and chick (Burr and Hovland '37; '37a).

To this end, a preliminary study was made on colonies of obelia collected at Provincetown, Cape Cod, from June 9 to 12, 1939. The measurements were made with the Burr-Lane-Nims ('36) microvoltmeter connected to the animals through silver-silver chloride electrodes immersed in salt solution.

With this technique it is possible to determine with considerable accuracy potential differences or voltage gradients between any two points on the animal to a large extent independent of changes in current and resistance.

In a number of preliminary determinations, one electrode was placed by means of a micro-manipulator on the stolon end of the cut hydrocaulus, and the other on the osculum of one of the distal animals of the colony. The potential difference here was of the order of 250 microvolts with the hydrocaulus positive. Further study showed, however, that a very large part of this potential difference was inherent in

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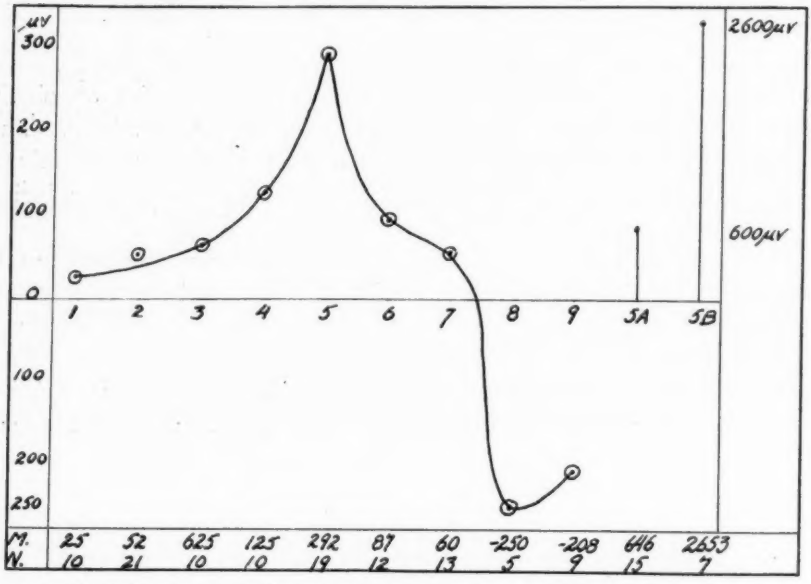
the specific animal, since if one electrode was placed upon the animal at the base of the hydrotheca where it joins the terminal annulation of the pedicel, and the other on the osculum, essentially the same voltage difference was given. In general the pedicels give approximately the same potential as the stolon end of the hydrocaulus. In other words the potential difference is constituted almost entirely within the animal as the colonial entity or individual; structurally and functionally apart from the pedicel on which it is borne and from which it springs. The distinction is shown in the pictures of Plate II.

Starting from this point, measurements were made on a series of stages of development in the animals. In all the measurements made there was such good agreement both within stages and between colonies that a very large number of determinations was not required.

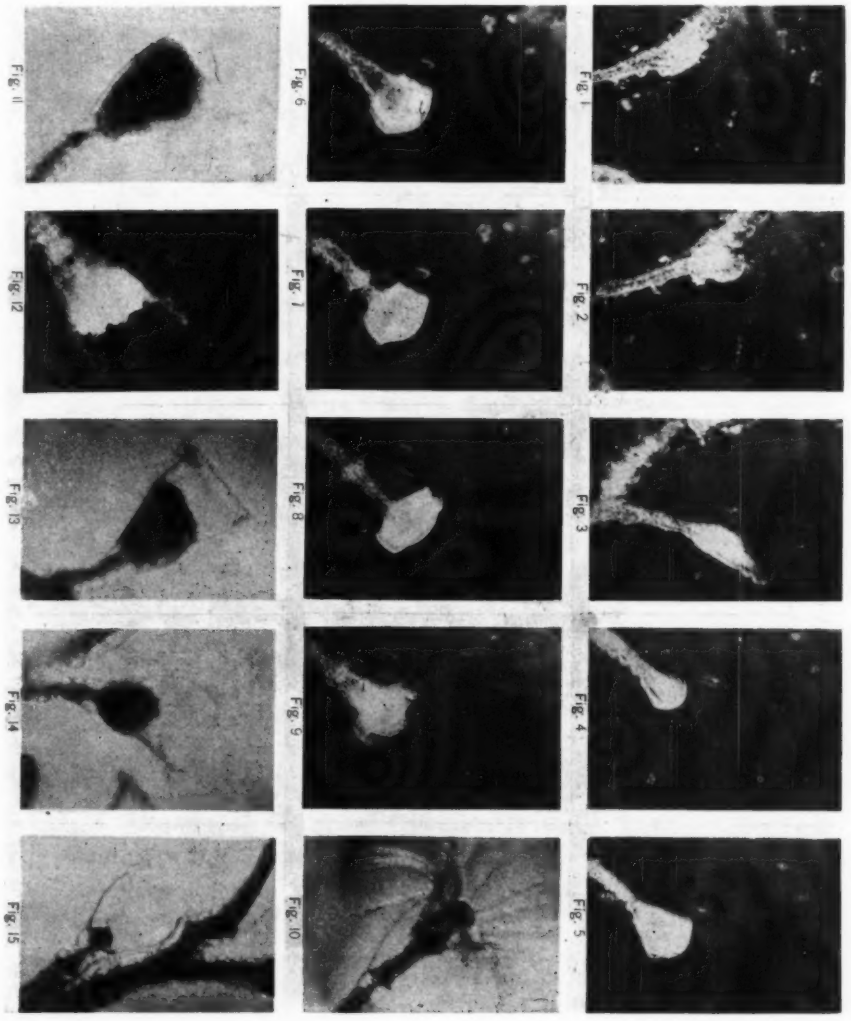
Plate I gives a graph of the varying gradients between the pedicel and the distal end of a series of stages of development of the hydranth and its regression to and through senility. The values plotted are the means of varying numbers of determinations for each stage.

PLATE I



A graph of the voltage gradients between the terminal annulation of the pedicel and the distal surface of definitive stages in the growth and life-cycle of obelia hydranths.

PLATE II



A series of photographs taken from the cinematic record of an obelia life-cycle showing the definitive stages thereof.

Stage 1 is the value obtained from the Anlagen of hydranth production as pictured in Plate II, fig. 1.

Stage 2 is the value obtained from the Bud as pictured in Plate II,

fig. 3. Bud production is a measure of new growth Initiation

Stage 3 is the value obtained from the Half developed animal as pictured in Plate II, fig. 5. Half stage formation is largely a matter of Proliferation.

Stage 4 is the value obtained from the Three-Quarter developed animal as pictured in Plate II, fig. 8. The Three-Quarter stage production is strongly factored by Differentiation.

Stage 5 is the value obtained from the Complete animal as pictured in Plate II, fig. 10. Organization is here significant. Up to here developmental growth and its accompanying anabolic increment has dominated.

Stage 6 is the value obtained as Regression sets in as pictured in Plate II, fig 11. Maintenance function has subsided.

Stage 7 is the value obtained from advanced Regression as pictured in Plate II, fig. 12. Here Maintenance has ceased.

Stage 8 is the value obtained when the animal is Senile as pictured in Plate II, fig. 14. Here catabolic dissolution is taking place.

Stage 9 is close to the final or Empty stage in the life cycle of an obelia hydranth. It is the stage which just precedes the formation of a new animal by Recurrence. It is represented in Plate II by fig 15.

These several stages are described in detail in other places (Hammett '35; '36; Hammett and Elliott '35).

It will be noted that the voltage gradient rises in a characteristic fashion during the course of developmental growth from the anlagen to the complete functional stage.

When function ceases the voltage gradient drops rapidly, then more slowly as regression continues, until suddenly, as senile dissolution takes over, the polarity of the gradient is reversed so that the distal surface of the dwindling animal becomes over 200 microvolts positive with respect to the base of the hydrotheca.

The smooth concordance of voltage gradient with the definitive ascending and descending phases of the life cycle of an obelia hydranth is highly significant. Significant in that it gives another demonstration of interrelation between potential and growth; significant because it allows tentative postulation of the potential basis; and significant because it gives another and striking confirmation of the postulate that senile dissolution is determined by an endogenous catabolism.

As the developmental growth of an obelia hydranth from its embryonic stage to that of a complete functionable animal increases, so increases the mass, the complexity, and the chemical intensity of the animal's reactions.

It is a nice question as to which of these increases is more largely responsible for the increase in voltage gradient.

There is much that might be said for increase in mass as the dominant factor. Weakening this idea however are the studies of the inside-outside gradients of the Complete animals. In the first series one electrode was placed on the hydrotheca and the other within the mouth; in the second the first electrode remained on the hydrotheca while the second was inserted into the enteron.

Between the cavity of the mouth and the outside of the hydrotheca, the average of 15 determinations showed a voltage gradient of 646 microvolts with the outside positive and the inside negative.

By careful manipulation it is possible to introduce the micropipette of the electrode into the enteron. Here the voltage gradient is quite high, the average of 7 determinations was 2653 microvolts; the maximum obtained was 3250 microvolts.

Something other than difference in mass potential would seem to be responsible for this 4-fold difference in voltage gradient.

Increase in structural complexity and diversity of action potentials also has questionable aspects as an explanation for the rising voltage gradient on growth. For Regression which in its beginning determines no great change in these attributes does condition a marked drop in voltage gradient from that existing in the fully functioning animal.

The inclination is to attribute in considerable degree the rising values to rising intensity of chemical reaction consequent upon the developmental unfolding.

Distinction is here rigidly made between chemical reaction capacity and chemical reaction intensity.

The former is primarily related to the amount of substance. The latter is primarily related to the kind of substance. The kind of substance at the completion of growth is not the kind of substance present at its beginning. The progressive developmental approach of an organism toward functionableness determines a progressive change in kind of substance which change involves an increasing acquisition

of ability to function. As the protoplasm of a developing organism becomes more and more constituted to take on its characterizing function, the chemical reactions which are to perform that function become more and more coordinated. Thus as the organism grows so does the intensity of functional faculty grow, and as the intensity of functional faculty grows so does the intensity of the constituent chemical reactions increase.

There is certain evidence besides the inferential to sustain the idea that intensity of chemical reaction is a significant participant in the voltage gradient rise on growth.

Thus the 4-fold greater gradient of the enteron as contrasted with the osculum is directly correlated with the greater chemical activity of the stomach as compared with the mouth, a difference which has been demonstrated in this animal by Hammett and Chapman ('38).

Conversely there is decrease in gradient with decrease in chemical activity as shown by the marked drop in potential when the animal ceases its feeding function and goes into physiological regression.

A third bit of evidence is available which is even nicer since it derives from the growing rather than the functioning or receding animal.

During one phase of the study it was noted that there was a good deal of fluctuation in the voltage gradient obtained from two electrodes placed upon a Half hydranth as it was passing into the Three-Quarter. These fluctuations consisted of fast and slow waves, varying from 100 to 700 microvolts in magnitude. As one of us (F. S. H.) has demonstrated, this is a period during which differentiation is getting established. The course of this developmental phase when observed under the microscope is not one of undeviating rectitude but consists of a series of advances and regressions with the successive advances bringing the animal more towards the Three-Quarter stage and the recessions less to the Half until the animal finally makes up its mind to settle down as a structurally differentiated organism. A cinematic record of the complete growth of a single animal from its anlagen stage to that of complete functioning was made something over a year ago. This record shows the advances and recessions perfectly and a portion of the film in which such a cycle is pictured is given in Plate III, fig. 1. The change from a young Three-Quarter stage back to a late Half and then again on to a later Three-Quarter as evidenced by the distal-ventral narrowing of the terminal portion took place during

PLATE III

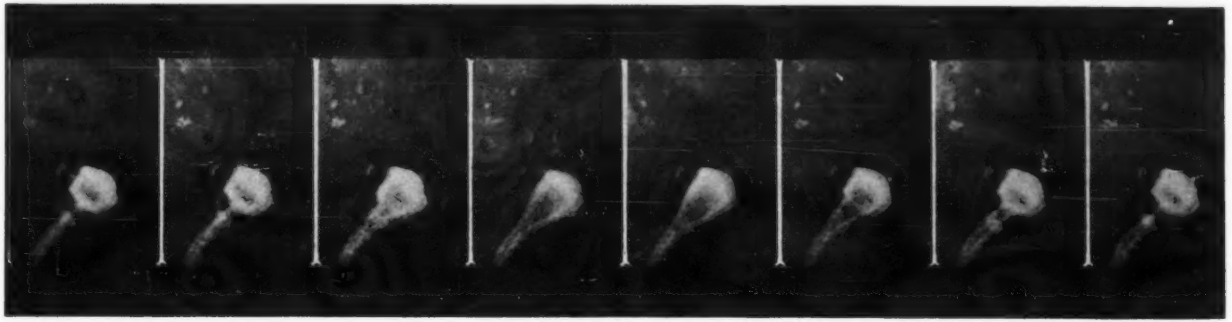


FIGURE 1

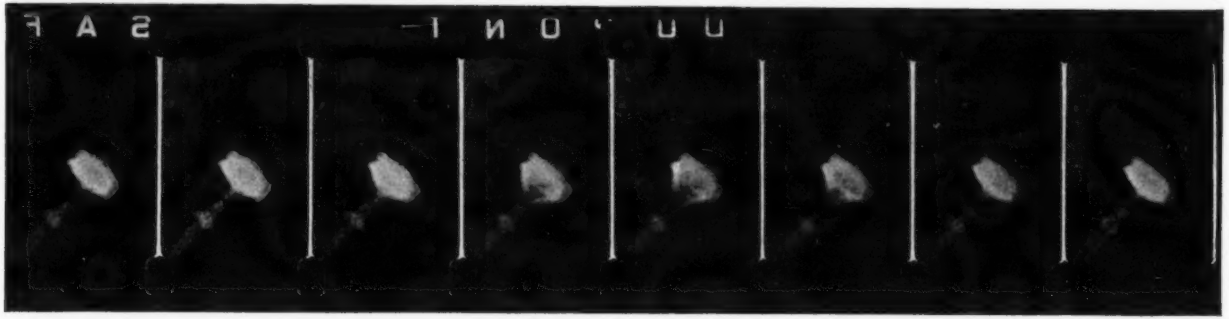


FIGURE 2
Cinematic record of the developmental fluctuations in structural differentiation and organization of *Obelia geniculata*.

120 seconds. The single shots were taken every 15 seconds.

This is actually a period of great developmental activity. The fact that microscope observations through many years, the cinematic record, and the voltage gradient agree with respect to the phenomenon of fluctuation would suggest that voltage gradients and the changes in intensity of chemical reactions concerned in the growth reactions are closely correlated.

Support for the belief is given by the fact that similar though much less definite or marked electrical fluctuation accompanied the developmental fluctuations which occur when a Three-Quarter is trying to become a Complete. The cinematic record of one of the late cycles in this attempt is shown in fig. 2, Plate III. It is to be noted that fluctuation is much less in extent than that which obtained in the case of the change from the Half to the Three-Quarter stage. Nevertheless the segregation of organization like the specialization of differentiation proceeds in fluctuating character.

Growth progression from the Anlagen and Bud stages shows no such marked structural fluctuations nor was evidence given of electrical variance. The concordance of no electrical fluctuation with no growth fluctuation; and of electrical fluctuation with growth fluctuation strengthens the belief in the validity of the association. And well there should be for it is but to be expected that such growth changes as are seen here should have their accompanying voltage gradient changes.

On the other hand here and there in the cinematic record there are rhythms which are associated with the early stages of structural growth. These hints and the findings reported above are suggestive enough to make it eminently worth while to investigate the entire cycle much more rigorously, and if possible to record simultaneously by means of moving picture technique the morphological and the electrical changes. Such a procedure would be full of technical difficulties and would involve the expenditure of considerable time and money.

While the results reported here represent a very small beginning in this problem, the internal consistency of the results is so high, it is doubtful if added numbers would materially change the essential picture. Nevertheless it is proposed to continue these studies on a more extensive scale as soon as possible.

The most striking event of the present study is the reversal of voltage

gradient which follows the physiological regression of the complete feeding hydranth. This regression is a shrinkage into the hydrotheca. Plate III. fig. 12. The tentacles, osculum, and other organs lose their identity. They seem to undergo an "Einschmelzung" into an amorphous mass which occupies about ⅔ of the hydrotheca. Plate III, fig. 13. This change is irreversible. The animal in this state is called Senile. The senile animal is thus the intermediate stage between the complete feeding organism and the past structure where nothing remains but an empty hydrotheca. Plate III, fig. 15.

This change from the senile to the empty stage is due to catabolism. The evidence is,—first there is gradual shrinkage and disappearance of the substance of the senile organism; second no loss of substance outwards into the culture solution is apparent, but rather a thinning from within; the third the pedicular hydroplasm, instead of streaming towards the disintegrating animal, as it does towards the growing one, streams away and back into the colonial hydrocaulus. It is not unreasonable to assume that this reversal of direction of hydroplasmic streaming means dissolved material is being carried away, just as it can be assumed that the streaming towards the growing animal is evidence that substance to be used for tissue building is being carried thereto (Hammett and Elliott '35). These observations and conclusions regarding the catabolic nature of the senile dissolution have been confirmed and sustained *in toto* (Hammett and Padis '35: Hammett and Chapman '38).

Now the processes of growth are accumulative while those of senility are disintegrative. The voltage gradient is in one direction when the accumulative processes predominate; in the other when the disintegrative are to the fore. The endogenous reversal is paralleled by voltage gradient reversal. This perfect concordance between voltage gradient direction and life-cycle direction substantiates the interpretation placed upon these electrical changes. Adding significance to this is the fact that when dissolution is essentially over and the rest period about to begin preparatory to production of a new hydranth by Recurrence an upturn of the voltage gradient curve towards the neutral position is produced. This is shown in Plate I, stage 9, and Plate II, fig. 15.

The reversal in voltage gradient cannot be due to reversal in direction of hydroplasmic streaming since no such electrical change is seen in the complete feeding animal where current flow is now in one

direction; now in the other. It can only be due to reversal in type of chemical processes. It is thus at one and the same time a proof the processes concerned in the natural disappearance of an obelia hydranth are the opposite of those concerned in its growth, i. e. that they are endogenously catabolic; and a demonstration that the electrical variance recorded in these studies is a valid index of the state of growth and life-cycle progression.

SUMMARY AND CONCLUSION

A series of measurements of the voltage gradients of definitive stages in the life-cycle of an obelia hydranth has been made.

These trace a rising curve of gradient intensity parallel with the course of developmental growth from anlagen to the complete functionable state. The values reach their peak in the feeding animal and then drop off as regression to the senile state begins. With the attainment of senility and its consequent catabolic dissolution the direction of the voltage gradient is reversed. Fluctuations in voltage gradient occur parallel with fluctuations in developmental growth progression from the undifferentiated to the differentiated state.

The conclusion is that the growth and life-cycle of an obelia hydranth is characterized by definitive and progressive changes in voltage gradient.

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